MORPHO-PHYSIOLOGICAL, YIELD AND OXIDATIVE STRESS RESPONSES OF SESAME UNDER WATERLOGGING STRESS

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*This is to certify that thesis entitled, "**Morpho-physiological, yield and oxidative stress responses of sesame under waterlogging stress**" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE in AGRONOMY**, embodies the result of a piece of bona fide research work carried out by **Taufika Islam Anee**, Registration No. **10-03890** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

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MORPHO-PHYSIOLOGICAL, YIELD AND OXIDATIVE STRESS RESPONSES OF SESAME UNDER WATERLOGGING STRESS

ABSTRACT

Waterlogging is one of the most devastating abiotic stresses which often results in oxidative stress to plants. Sesame (*Sesamum indicum* L. cv. BARI Til-4) is extremely susceptible to anaerobic environment induced by waterlogging and the hypoxic/anoxic condition which results in the reduced growth and yield along with the overproduction of various reactive oxygen species (ROS; $O_2^{\bullet-}$, $OH^{\bullet}$, H_2O_2 and $^1O_2$). The present study was designed to investigate the duration-dependent changes in the morpho-physiological, anatomical and biochemical attributes of sesame to waterlogging stress. The sesame plants were subjected to waterlogging for 2, 4 and 6 days at vegetative, reproductive and maturity stage of plant growth and the data were measured after completion of the each treatment duration. A treatment with 8 days waterlogging duration was added for the biochemical study. Present study proves that waterlogging exerts severe damage effect on different physiological parameters and yield attributes of sesame plant. The plants showed an increasing trend in the lipid peroxidation, H_2O_2 and methylglyoxal (MG) content with the increasing duration of stress. Proline (Pro) content was slightly decreased along with the leaf relative water content (RWC) with increasing duration of waterlogging. Photosynthetic pigments like chlorophyll *a*, *b*, and total chlorophyll and carotenoid contents also decreased with time in stressed plants. The content of glutathione (GSH) and oxidized glutathione (GSSG) increased, while the ratio of GSH/GSSG and Ascorbate (AsA) content decreased which indicates the the disruption of redox balance in the cell. Activities of ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR) and glutathione peroxidase (GPX) increased, while the dehydroascorbate reductase (DHAR), glutathione reductase (GR) and catalase (CAT) activities mostly decreased. Waterlogging modulated the glyoxalase system mostly by enhancing glyoxalse II (Gly II) activities with a slight increase in glyoxalse I (Gly I). So, the present study also proves the induction of oxidative stress under waterlogging stress in sesame plants and the enhancement of the stress level with increasing duration.

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LIST OF ABBREVIATIONS

ADH	Alcohol dehydrogenase
AO	Ascorbate oxidase
APX	Ascorbate peroxidase
AsA	Ascorbic acid/Ascorbate
ATP	Adenosine triphosphate
BARI	Bangladesh Agricultural Research Institute
CAT	Catalase
Chl	Chlorophyll
DAE	Department of Agricultural Extension
DAS	Days after sowing
DHAR	Dehydroascorbate reductase
DW	Dry weight
FAO	Food and Agriculture Organization
FL	Flooding
FW	Fresh weight
Gly I	Glyoxalase I
Gly II	Glyoxalase II
GPX	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
GST	Glutathione *S*-tranferase
HI	Harvest index
LDH	Lactate dehydrogenase
MDA	Malondialdehyde
MDHA	Monodehydroascorbate
MDHAR	Monodehydroascorbate reductase
MG	Methylglyoxal
MSI	Membrane stability index
NADPH	Nicotinamide adenine dinucleotide phosphate
PAR	Photosynthetically active radiation
PDC	Pyruvate decarboxylase

LIST OF ABBREVIATIONS (Cont'd)

POD	Peroxidase
POX	Peroxidases
Pro	Proline
PSI	Photosystem I
PSII	Photosystem II
ROS	Reactive oxygen species
RuBisCo	Ribulose-1,5-bisphosphate carboxylase/oxygenase
RWC	Relative water content
SOD	Superoxide dismutase
SRDI	Soil Resource Development Institute
WL	Waterlogging

Chapter 1

INTRODUCTION

Sesame (*Sesamum indicum* L.) is considered to be the most ancient oilseed crop known and domesticated by farmers for more than 5000 years ago (Langham, 2007). Sesame belongs to the family Pedaliaceae. It is a self-pollinating plant with an erect, pubescent and branching stem. It is grown widely in both tropical and subtropical regions (Ashri, 2010). India, Sudan, China, Mayanmar and Nigeria are the top sesame producing countries and China and Japan are the leading sesame importers (FAO, 2014). Though it ranks ninth among the existing oil seed crops of the world (Kafiriti and Mponda, 2013), it has gain much popularity due to its long durability, easy extraction, low rancidity and drought resistance. It has both nutritional and medicinal values (Morris, 2002; Wu *et al.,* 2006; Kanu *et al.,* 2010; Bopitiya and Madhujith, 2013). It not only serves as human food (Morris, 2002) but also as animal feed (Mukhopadhyay and Ray, 1999) in the form of oil, whole seed or meal etc. (Hahm *et al.,* 2009). Sesame contains 42-50% oil, 25% protein and 16-18% carbohydrate (Khan *et al.,* 2009). It is rich in fatty acids including oleic acid (41–45%), linoleic acid (37–42%), palmitic acid (10%) and stearic acid (5%) (Kang *et al.*, 2000; Kim *et al.,* 2002; Hall, 2003). Sesame seed also contains some lipid-soluble antioxidants such as sesamin, sesamolin, sesamol and sesaminol (Sirato-Yasumoto *et al.,* 2001) and some other components such as lignan glucosides, phytosterols, tocopherols etc. which have health promoting properties (Katsuzaki *et al.,* 1994; Shah idi *et al.,* 1997; Kato *et al.,* 1998; Coulman *et al.,* 2005; Hahm *et al.,* 2009; Kanu *et al.,* 2010).

The climate and soil conditions of Bangladesh are quite suitable for sesame cultivation. The crop is grown both in rabi and kharif seasons but the kharif season

covers about two-third of the total sesame area (Shuva *et al.,* 2006). As sesame is drought tolerant it can be grown successfully in the early summer (March-May) under rainfed condition. Sesame has the potential capacity to combat nutritional deficiencies in developing regions and countries (Wang *et al.,* 2016). In Bangladesh it is grown covering 1,03,000 ha area with an average yield of 0.96 t ha^{-1} (DAE, 2014). It is the second largest source of edible oil seed crop in Bangladesh. The main regions of our country growing sesame are greater Faridpur, Barisal, Rangamati, Dinajpur, Pabna, Khulna, Dhaka, Mymensingh, Comilla, Rajshahi, Jessore, Patuakhali, Rangpur and Sylhet (Pathan *et al.,* 2007; Shuva *et al.,* 2006). The growth and yield of sesame is limited by low genetic yield potential and by biotic and abiotic factors (Jyothi *et al.,* 2011; Radhakrishnan *et al.,* 2014). The yield of this crop in Bangladesh is found lower compared to that in other countries (Khan *et al.,* 2009).

Waterlogging is one of the most devastating forms of abiotic stress. Due to erratic pattern of rainfall and other extreme climate events, waterlogging or flooding has now become a major threat for crop production and desired yield. Several anthropogenic causes i.e., improper drainage practices, failure of dame – are also sometimes responsible for this unwanted occurrence of flood which further leads to waterlogged conditions (Hasanuzzaman *et al.,* 2012a). Bangladesh is vulnerable to flood due to presence of a monsoon season which causes heavy rainfall. Other factors which have contributed to flooding are deforestation which causes soil erosion. This leads to the increased silt content in the rivers further downstream. This decreases the carrying capacity of the rivers, causing the peak flow of the river to increase (NDA, 2016).

Waterlogging decreases the available O_2 for plants (Capon *et al.,* 2009). Water logging stress starts with the hypoxic (deficiency of O_2) stress limiting mitochondrial

respiration. With the succession of time it creates anoxic (absence of O_2) stress which results in inhibition of respiration (Wegner, 2010). Under waterlogging condition due to oxygen deficiency and low light intensity reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$) are overproduced which are responsible for photooxidative damage (Yordanova *et al.,* 2004). Together with altered physiological and growth processes, waterlogging severely affects the reproductive development which causes yield reduction as reported in a number of plant species.

Sesame is extremely susceptible to waterlogging and continuous heavy rains. When grown on soils with poor drainage, sesame is adversely affected and can suffer yield losses of greater than 30% (in severe cases, 50–90%) (Zhang *et al.,* 2012). Field experiments on waterlogged sesame have recorded premature senescence resulting from leaf chlorosis, necrosis, defoliation, and reduced nitrogen fixation, leading to cessation of growth and reduced yield (Snowden and Wheeler, 1993). Waterlogging induced oxidative stress and antioxidant enzyme activities along with the formation of distinct aerenchyma have also been reported in sesame (Wei *et al.,* 2013).

Considering the circumstances stated above and based on the available literatures this study was undertaken with following objectives:

i. to investigate the waterlogging-induced morpho-physiological and yield damages in sesame plants,
ii. to evaluate the most susceptible stage of sesame under waterlogging stress,
iii. to measure the changes in oxidative stress markers and antioxidant defense system with different duration of waterlogging.

Chapter 2

REVIEW OF LITERATURE

2.1 Sesame

Sesame is a survivor crop. For 5,000 years it has been planted by subsistence farmers in areas that will not support the growth of other crops or under very difficult conditions with drought and/or high temperature. In some countries it is grown after the monsoon on residual moisture with no rains during its production cycle. In some countries it is grown in the monsoon season and subject to daily rains during parts of its cycle. In several countries it is the last crop that can be grown at the edge of deserts, where no crops grow. Very little sesame is grown under high input conditions, although yields improve dramatically as inputs increase (Langham, 2007). Unfortunately, the major obstacle to sesame expansion is low seed yield which results due to lack of non-shattering, waterlogged, and disease & insect resistant variety. The genus *Sesamum* has many species, mostly wild which are recorded to be native to sub-Saharan Africa and the cultivated type species *Sesamum indicum* is originated in India. The Oilseed Research Centre of Bangladesh Agricultural Research Institute (BARI) has released four improved varieties of sesame, of which the first variety was Til-6 which was released in 1976 and last variety named BARI Til 4 was released in 2009. These varieties are late in maturity and very much susceptible to excess water. Due to high competition among different high valued crops over the years, the acreage and production of sesame have dramatically decreased (Miah *et al.*, 2015). Moreover, with the increased frequencies of extreme climate events like cyclone, flash flood and heavy rainfall, the survival of this flood susceptible crop has come to a at stake. There are many studies reporting the effect of different waterlogging periods and regimes on sesame growth and yield. But, research works related to the oxidative

stress caused by waterlogging and the related enzymatic activities are very limited in number. However, some of the research findings which are very relevant to our study and provided useful information are reviewed in this chapter.

2.2 Abiotic stress

Generally crops are grown under field conditions and in uncontrolled environment where crops may frequently get exposed to different abiotic stresses. The unpredictable conditions of environment and moreover the intricate features of nature are leading to frequent changes in global climate, which results in adverse situation for crop (Mittler and Blumwald, 2010). A number of abnormal environmental parameters are collectively termed abiotic stress which includes: salinity, drought, extreme temperatures (high/low), flooding/waterlogging, heavy metal or metalloids, nutrient deficiencies, high/low light, UV-radiation, ozone etc. Plant growth, physiology, metabolism and productivity- every aspects of crop production are negatively affected by abiotic stress conditions. If the stress becomes very high and/or continues for an extended period it may lead to an intolerable metabolic load on cells, reducing growth, and in severe cases, result in plant death (Hasanuzzaman *et al*., 2012a). However, the magnitude of plant stress varies depending upon the types of stressor and on the prevailing duration. In nature, plants may not be completely free from abiotic stresses. They are expected to experience some degree of stress by any factor(s). Some environmental factors, such as air temperature, can become stressful in just a few minutes; others, such as soil water content, may take days to weeks, and factors such as soil mineral deficiencies can take months to become stressful (Taiz and Zeiger, 2006).

2.3 Waterlogging stress

Waterlogging has been defined as "the state of land in which the subsoil water table is located at or near the surface with the result that the yield of crops commonly grown on it is reduced well below for the land, or, if the land is not cultivated, it cannot be put to its normal use because of the high subsoil water table" (FAO, 2015). Waterlogging or flooding has harmful impacts on worldwide agricultural productivity. Reduction of the concentrations of cellular oxygen and of carbon dioxide in root surroundings are caused due to flooding induced water superfluity, as a result plants face injurious damages (Jackson *et al.,* 2009). Waterlogging reduces soil redox potential and enhances generation of toxic compounds including sulfides, soluble iron (Fe) and manganese (Mn), ethanol, lactic acid, acetaldehyde and acetic and formic acid (Fiedler *et al.,* 2007). Alteration in root hydraulic conductivity, light interception, stomatal conductance, CO_2 assimilation, drastic reduction of photosynthesis, alteration in respiration, generation of a range of secondary metabolites are responsible for reducing plant potential to grow and showing their productivity under waterlogging stress (Ashraf, 2012). Inhibitions of respiration together with generation of toxic compounds potentially hamper the metabolic processes of plants growing under waterlogging condition. Stunted growth and reduced biomass have been reported in plants growing under waterlogged environment. River floodplains and coastal areas are the most prone areas to flooding; however, it is also possible for flooding to occur in areas with unusually long periods of heavy rainfall. Flooding stress alone can be claimed to reduce yield up to 50% in different crops (Bhushan *et al.*, 2007).

2.4 Effect of waterlogging on crop attributes

2.4.1 Effect on growth

Reduction of growth rate is one of the major and initial effects of waterlogging stress. Waterlogging condition dramatically affect plant growth, development and survival in various ways (Parent *et al.*, 2008).

An experiment was conducted by Ali *et al.* (1999) with different *Zea Mays* L. cultivars. They observed that the height and diameter of plant, number of leaf and total life period were harshly affected by waterlogging (2 cm standing water).

In order to study the effects of different submergence regimes on *Phragmites australis* plant Mauchamp *et al.* (2001) conducted an experiment and observed that partial submergence (50 and 80%) considerably improved accumulation of biomass and growth, while full submergence reduced the values of same parameters. Moreover, 18.7% mortality rate was recorded in case of full submergence of 40-day-old seedlings.

Waterlogging stress was reported to reduce specific leaf area and leaf areas by 26 and 36%, respectively and increased specific leaf weight by 22% in contrast to control in Quinoa (*Chenopodium quinoa* Wild.) plants (González *et al.*, 2009). They also observed lower dry weight of root, stem and leaf in waterlogged plants.

In maize, plant height and ear height were severely affected and in almost all genotypes plant height reduced due to flooding treatment. At knee height stage, responses to excess soil moisture in different genotypes showed fair correspondence with the responses at seedling. Flooding at knee height stage of the crop growth resulted in immediate wilting of plants starting from the base of the plant and

subsequent lodging of most of the plants. In leaves, wilting started from the tip and proceeded towards the base of the leaf (Lone and Warsi, 2009).

A greenhouse experiment was carried out by Ezin *et al.* (2010) who investigated the effect of flooding on two tomato cultivars and two wild related species. Different durations (2, 4 and 8 days) of flooding were imposed to 40-days-old tomato plants which exerted harmful impact on plant height, length of leaves, leaf number and enhanced the formation of adventitious root in all cultivars. Among the tomato genotypes LA1579 showed sensitiveness to flood. The CLN2498E and CA4 genotypes exhibited high tolerance to flooding stress whilst LA1421 genotype showed lower tolerance.

The performance of two chickpea cultivars in well-drained and subsurface waterlogging conditions was compared during their vegetative growth stage. It was observed that the waterlogging reduced the leaf area by about 70% for both chickpea cultivars (Deshi cultivar; Rupali and Kabuli cultivar; Almaz). Shoot dry matter accumulation under waterlogging condition reduced by 70% in Rupali cultivar and it reduced by 56% in Almaz cultivar. The number of branches decreased by 50% in both chickpea cultivars under waterlogging stress. As a result, shoot dry matter accumulation decreased significantly. Subsurface waterlogging altered the rooting pattern in chickpea, inhibited the branching of root and the growth of tap root and consequently disturbed the overall growth of root (Paltaa *et al.,* 2010).

Tolerant and sensitive type of mung bean (*Vigna radiata)* genotypes including viz., T 44 and MH–96–1 (tolerant) and Pusa Baisakhi and MH–1K–24 (sensitive) were experimented by Kumar *et al.* (2013) for waterlogging induced changes. Thirty-day-old plants were waterlogged for 3, 6 and 9 days. They observed that waterlogging

reduced the leaf area, crop growth rate, root growth and nodules number in all mung bean plants where tolerant plants showed lower reduction of these parameters.

In green gram cultivars, under waterlogging condition Prasanna and Rao (2014) reported significant difference of the growth parameters. Plant height, leaf area, number of leaves and total dry matter were significantly affected by waterlogging throughout the life cycle. The effect of 4 days waterlogging was more severe in comparison with 2 days waterlogging treatment over the control. Waterlogging reduced the plant height by 33%, number of branches by 34%, number of leaves by 31%, leaf area by 31% and total dry matter by 30%.

Different stages of summer maize were studied under waterlogging condition by Ren *et al.* (2014). In field, the created waterlogging for different durations (3 and 6 days) at the three-leaf stage (V3), six-leaf stage (V6), and the 10^{th} day after the tasseling stage (10VT) of maize. The results after 2 years of study indicated that waterlogging affected the overall growth and development of summer maize negatively.

2.4.2 Effect on plant physiology and metabolism

Physiology and metabolism of plants are disrupted under waterlogging condition. Plants show a number of responses including hampered stomatal conductance, net CO_2-assimilation rate and root hydraulic conductivity (Ashraf, 2012) along with reduced photosynthetic rate (Akhtar and Nazir, 2013) under waterlogged condition.

In the study of Lone and Warsi (2009) with *Zea mays* L., it was observed that waterlogging stress had a great impact on few of the physiological traits like photosynthetically active radiations (PAR) and transpiration rate of maize crop except

leaf temperature which remained mostly constant under both normal and waterlogged conditions and in both winter and summer season.

Waterlogging negatively affected the photosynthetic rate of *Triticum aestivum* L. which was mainly attributed to leaf chlorosis and waste in harvested energy by the PSII reaction center dispersed via non-photochemical approaches (Zheng *et al.,* 2009).

Liao and Lin (1996) carried out an experiment with *Momordica charantia* L. and observed that flooding stress decreased the transpiration rate, leaf photosynthetic rate, stomatal conductance, activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), and soluble protein compared with control seedlings.

Reduction of relative water content (RWC) and membrane stability index (MSI) were reported in all the tested genotypes of mung bean with the progress of waterlogging. Under waterlogging condition, tolerant genotypes (T-44 and MH-96-1) upholded considerably higher RWC and MSI than the sensitive genotypes (MH-1K-24 and Pusa Baisakhi). Photosynthesis rate decreased in all tested genotypes under waterlogging stress and these inhibitions increased in duration dependant manner. Like photosynthesis rate, stomatal conductance also exhibited the same trend. In comparison with control plants, respiration of leaf increased after 48 hrs of waterlogging. MH-96-1 and Pusa Baisakhi showed higher leaf respiration rate than the other genotypes. Minor reduction of respiration rate was recorded at 6^{th} and 9^{th} d of waterlogging. But, leaf respiration rate in T-44 remained unaltered throughout the period of waterlogging treatment. Therefore they concluded that respiration of leaf did not reduce due to any tested duration of waterlogging (Kumar *et al.,* 2013).

The detrimental effect of waterlogging on water loving plants has also been reported in some studies. Anandana *et al.* (2015) recorded some damages in rice plant under long time flooding stress. *Oryza sativa* cv. Puzhuthiikar showed notable enhancement of leaf blade length, sheath length and area but reduction of leaf blade area. Accordingly, transpiration, rate of photosynthesis and intercellular CO_2 was augmented in rice plant.

Pezeshki (2001) reported wetland vegetations acquire diverse characteristics that facilitate them to stay alive under sporadic soil saturation and the associated changes in chemistry of soil. These alterations comprise the decreasing of soil redox potential (Eh) which transform into a gradually greater demand for oxygen inside the soil and therefore extra pressure on the plant roots. On the other hand, data on the link between flood-response of swamp/wetland plants and reducing soil situations is very limited. Particularly, the link between reduction of soil and photosynthesis of plant is basically unknown, but the literatures disclose a variety of sensitivities in photosynthesis to the intensity of soil reduction among the wetland species. Preliminary lessening in net photosynthesis instantaneously after flooding in soil is very common among species representing diverse wetland ecosystems including forest, riparian and marshes wetlands. During the full life cycle of plants, the photosynthetic reduction is documented to diffusional restrictions due to closure of stomata and to metabolic (non-stomatal) inhibition of photosynthesis. Low soil redox potential (Eh) may possibly direct to photosynthetic reduction due to reduced leaf water potential resulting from dysfunction of root, reduced activity of key photosynthetic enzymes, disturbance in transport of photosynthate, changes in source-sink relationship or reduced sink demand. Moreover, whilst root oxygen-deficiency might moderately account for the reduction in net photosynthesis, soil phytotoxins

formed as by-products of low soil Eh situations may play a key function in the pragmatic sensitivities in photosynthesis. Undoubtedly, intense reduction in soil elevated oxygen demand which exerts insightful influence on transportation and release of oxygen to the rhizosphere.

In, *Tamarix ramosissima* plants stomatal conductance, photosynthesis rates, transpiration, internal (intercellular) CO_2, and activity of root alcohol dehydrogenase (ADH) in *T. ramosissima* under different types of soil under flooded condition or non-flooded condition were compared. Photosynthesis at 1500 mol quanta m^{-2} s^{-1} (A1500) in flooded vegetations ranged from 2.3 to 6.2 mol CO_2 m^{-2} s^{-1} during the first week, but A1500 enhanced to 6.4–12.7 mol CO_2 m^{-2} s^{-1} by the third week of flooding. Stomatal conductance (g_s) at 1500 mol quanta m^{-2} s^{-1} also reduced primarily because of flooding, where g_s was 0.018 to 0.099 mol H_2O m^{-2} s^{-1} during the first week, but gs increased to 0.113–0.248 mol H_2O m^{-2} s^{-1} by the 3rd week of flood stress. Conversely, photosynthesis of plants under flooded condition decreased by non-stomatal limitations, and subsequent augmentation indicated metabolic acclimation to flooding. Flooded condition increased ADH activity of plant compared to control or drained plant which indicating oxygen stress. Higher oxygen stress and lower photosynthesis rate made the plant more susceptible to flooding. However, type of soil had no significant effect on photosynthesis or on root ADH activity. In the field condition, leaf water potential, transpiration, stomatal conductance, and leaf $\delta^{13}C$ were compared between *T. ramosissima* and other flooded species. *T. ramosissima* had lower stomatal conductance and water potential compared to *Phragmites australis* and *Populus deltoides*. Differences in physiological responses for *T. ramosissima* could become important for ecological concerns (Polacik and Maricle, 2013).

Flooding stress enhanced the number of leaves but decreased the photosynthetic activity in *Cichorium intybus* plant by closing stomata and reducing the efficiency of PSII. The roots of waterlogged plants became shorter compared to control plants. However, no significant differences were observed regarding fresh weight and dry weight. Roots of flooded plant accumulated glucose, fructose, sucrose and 1-kestotriose but leaves accumulated organic acids and reducing sugars. Activities of invertase and sucrose synthase increased in both leaf and root while the activity of sucrose-phosphate-synthase remained unaltered due to flood stress. Synthesis of inulin was delayed in roots of flooded plants and its mean degree of polymerization decreased as a consequence of inhibition of fructan: fructan 1-fructosyltransferase (Vandoorne *et al.,* 2014).

2.4.3 Effect on plant anatomy

Plants exhibit several anatomical changes under flooding/waterlogging condition. Under flooding/waterlogging condition, plants initiate adventitious root, hypertrophied lenticel and/or aerenchyma (Ashraf, 2012) for adjusting with the unfavorable condition. It is thought that lenticels of plant are involved in the downward diffusion of oxygen or O_2 as well as, different by-products of anaerobic metabolism (ethanol, CO_2 and CH_4) inside the plants. But, the concrete physiological function of lenticels is still ambiguous; their existence is often connected to waterlogging tolerance in plants (Parelle *et al.,* 2006).

Vasellati *et al.* (2001) conducted an experiment with *Paspalum dilatatum* plants to elucidate the anatomical changes of plants under flooding condition. They examined the constitutive and plastic anatomical traits of *P. dilatatum* populations from its original habitats with contrasting flooding regimes. Flooding enhanced the

aerenchymatous tissue in the root cortex and the leaf sheaths and reduced the number of root hairs per unit of root length. In addition to these plastic responses, all clones showed constitutive characteristics that may present a capability to hold out impulsive dealings of flooding. In *P. dilatatum* a high proportion of aerenchymatous tissue may maintain aeration before plastic responses take place; sclerenchyma tissue, which may resist root and leaf sheath collapse by soil compaction; and a conspicuous endodermis, which may protect stelar tissues from desiccation. Both constitutive and plastic anatomical characteristics in *P. dilatatum* are likely to contribute to the ability of this species to occupy widely diverse topographic positions and to resist temporal variations in water and oxygen availability.

But on the other hand, root cortex of flooded *Garcinia brasiliensis* seedlings did not show significant difference. However, significant differences were recorded in exodermis width between the two types of treatments. Under flooding condition, the exodermis enhanced the width by 24% in comparison to non-flooded plants. The proportion of aerenchyma in root cortex under flooded and non-flooded treatments did not show any statistical differences. Flooded condition significantly increased the width of phloem tissue. Correspondingly, number of xylem vessel in the roots of flooded seedlings also increased notably. On the other hand, area of xylem fibers decreased considerably under flooding condition (Corrêa de Souzaa *et al.,* 2013).

2.4.4 Effect on nutrient availability

Flooding/waterlogging can break the balance of availability of different essential nutrient in soil. So, flooding/waterlogging is known to create adverse effects on numerous biochemical and physiological processes of plants by inducing insufficiency of essential nutrients like N, K, Ca, Mg etc. (Ashraf, 2012).

Submergence led to enhanced N content of the rice plant tissues and it increased with the increase of submergence duration. Moreover, the submerged plants showed small amounts of P and K contents at all the three growth stages of the plant. Submergence resulted in reduction of carbohydrates production accompanied by proteins decomposition due to improved proteolytic activity. In addition deficiency of P and K hold back the synthesis of protein resulting in the accumulation of non-protein nitrogenous compounds. As a result tissue N content increased markedly in plant (Reddy and Mittra, 1985).

Atwell and Steer (1990) recorded that water stress decreased endogenous N, P, K levels in maize plant. Similarly Boem *et al.* (1996) demonstrated that short period of waterlogging stress significantly decreased the N, P, K and Ca uptake in canola plants.

In wheat, waterlogging significantly reduced the uptake of Zn, Cu, K and P in susceptible genotype in contrast to the tolerant genotypes (Tarekegne *et al.,* 2000). *Medicago sativa* showed a noticeable reduction in the nutrient composition (K, P, Cu, Ca, Mg, Zn and B) of leaf and root under waterlogging condition (Smethurst *et al.,* 2005).

2.4.5 Effect on yield

Except some water loving plants significant yield reduction occurs due to flooding or waterlogging. Flooding/waterlogging at any growth stage of plant ultimately hampers the yield of plant.

Reddy and Mittra (1985) conducted an experiment with rice plant and showed that grain yield was adversely affected by full submergence. Maximum yield reduction

(58%) was recorded from complete submergence at flowering stage. This reduction of grain yield was observed due to impaired anthesis as well as high sterility of flower. Around 69% unfilled spikelets were recorded in the experiment. Full submergence at seedling establishment stage reduced the grain yield by 29% and submergence at maximum tillering stage reduced the grain yield by 18% in rice plant.

Grain yield of maize showed drastic reduction under excess soil moisture conditions in both winter and summer seasons. In winter trial, overall yield was higher in all the genotypes in both sets of experiments but still yield loss (%) was higher. The yield reduction varies from 19% in YHPP45 (tolerant genotype) to 53% in Pop 3121 × YHPP45, While in case of summer trial, overall yields were lower but highest reduction in yield (66%) was detected in Tarun83 (susceptible genotype) and lowest reduction (2%) was detected in YHPP45 genotype (Lone and Warsi, 2009).

The reaction of four tomato genotypes was quite different from each other under waterlogging condition in the experiment conducted by Ezin *et al.* (2010). They found significant differences in days to flowering and days to maturity among the four genotypes under waterlogging condition. Flowering time and fruit set time was earlier in CLN2498E genotype than the genotypes CA4, LA1241, and LA1579. The average fruit weight in LA1421 and CA4 genotypes varied noticeably after 8 days of flooding, in contrast to control plants. No significant difference was observed in average fruit weight from CN2498E but slight reduction of fruit weight was observed with the increase of duration of flooding. Under flooding conditions, LA1579 genotype did not bear fruit from cluster 2 to 6. Compared to the control plants, total fruit weight from cluster 2 to 6 and the total yield from CLN2498E did not vary considerably. No significant reduction was observed up to 4 days of nonstop flooding in total fruit

weight from cluster 2 to 6 and total yield per plant in CA4 genotypes but 8 days of nonstop flooding significantly affected the total fruit weight from cluster 2 to 6 and total yield plant^{-1}. Total fruit weight and total yield of LA1421 genotype decreased significantly compared to the control plants under 2, 4, and 8 days of continuous flooding.

Paltaa *et al.* (2010) detected that the temporary waterlogging decreased the yield of seed by 54% in the Almaz (kabuli cultivar) and by 44% in the Rupali (desi cultivar). Kabuli cultivar Almaz showed reduction of seed yield resulted from 50% decline in the number of seeds pod^{-1}. However, Rupali (deshi cultivar) showed less pods number and number of seeds pod^{-1} under waterlogging condition.

Waterlogging imposition decreased grain yield in contrast to drained soils in the different genotypes of wheat plant (KRL 3-4, NW 1076, KRL 146, Brookton, PBW 343, KRL 200 and HD 2009) and higher reductions were recorded in sodic soils. Moreover, the reduction percentage varied differently in genotype to genotype. The reductions were 12% for KRL 3-4, 10% for NW 1076, 9% for KRL 146, 190% for Brookton, 162% for PBW 343, 3% for KRL 200 and 100% for HD 2009 in sodic soil, compared with genotypes grown under drained soils. This differential response of wheat genotypes might be due to the function of different tolerance mechanisms under waterlogging (Yaduvanshi *et al.,* 2010).

Similarly, Rasaei *et al.* (2012a) showed a significant difference among all the durations (10, 20 and 30 days) of the waterlogging stresses in wheat. Although, 30 days waterlogging had more negative effects on the grain yield, the period of 10 and 20 days waterlogging could also make a significant difference with non-waterlogging condition. The yield of wheat under non-waterlogging condition, 10, 20 and 30 days

of waterlogging were 7518.4 kg ha^{-1}, 6815.5 kg ha^{-1}, 5587 kg ha^{-1} and 4138.6 kg ha^{-1} respectively.

Kumar *et al.* (2013) carried an experiment with different genotypes of mung bean under waterlogging stress and found that yield was affected by waterlogging in all the genotypes. At vegetative stage yield loss enhanced with duration dependent manner. In average, grain yield losses in all mung bean cultivars were 20.01, 33.79 and 52 % due to 3, 6 and 9 days of waterlogging, respectively. The tolerant genotypes were able to recover the grain yield losses caused by 3 days waterlogging. On the other hand, for sensitive genotypes even 3 days waterlogging decreased the yield (upto 20%). In sensitive genotypes, grain yield losses were estimated 70.0 (Pusa Baisakhi) to 85% (MH–1K–24) after 9 days of waterlogging in comparison with control plants. Tolerant genotypes confirmed comparatively lower yield reduction even after 9 days of waterlogging.

Flooding stress was applied to six genotypes of wheat for 28 days and Amri *et al.* (2014) noted that the grain yield affected significantly in all evaluated genotypes. Compared to rainfed conditions (control), waterlogging induced an average decrease of grain yield by 56% with a maximum of 74% recorded for cv. Ariana and cv. Vaga against a lowest decrease of 39% recorded for the cultivars Salammbô and Utique. The two cultivars FxA and Haïdra showed an intermediate behavior with respective decreases of 60% and 48%.

Prasanna and Rao (2014) conducted an experiment with waterlogging in green gram. They observed that the waterlogging condition for 2 days and 4 days considerably decreased the yield components and finally yield. The number of pods $plant^{-1}$, number of seeds pod^{-1}, 100 seeds weight, harvest index and yield of green gram

decreased by 51, 27, 3, 33 and 71%, respectively due to 4 days of waterlogging and by 29, 14, 1, 13 and 25%, respectively due to 2 days of waterlogging in contrast to control plants.

An experiment was performed by Ren *et al.* (2014) in the field for studying the effects of waterlogging for different durations (3 and 6 days) on the yield and growth of summer maize at the three-leaf stage (V3), six-leaf stage (V6), and the 10^{th} day after the tasseling stage (10VT). The results after 2 year indicated that maize development and grain yield responses to waterlogging depended on both stress severity (intensity and duration) and different growth stages. Yield decreased significantly with an increased waterlogging duration during V3 and V6. The yields of maize hybrid Denghai 605 (DH605) in treatments V3-3, V3-6, V6-3, V6-6, 10VT-3, and 10VT-6 were 23, 32, 20, 24, 8, and 18% lower than those of the control (CK), respectively; Yields of Zhengdan 958 (ZD958) were decreased by 21, 35, 15, 33, 7, and 12%, respectively, compared to control.

2.5 Waterlogging induced oxidative stress and antioxidant defense system

2.5.1 Oxidative stress under waterlogged condition

Waterlogging, as an abiotic stress also provokes the production of reactive oxygen species (ROS) in different forms and different subcellular compartments (Jaspers and Kangasjärvi, 2010). These ROS include both free radicals (superoxide radical, $O_2^{\bullet-}$ and hydroxyl radical, $OH^{\bullet}$) and molecules such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) (Blokhina *et al.,* 2003). When a plant or plant part either enters to hypoxia/ anoxia from normoxic conditions or returns to an aerobic environment ROS are produced in transition (Irfan *et al.,* 2010). These ROS if generated in larger quantity may oxidize proteins, lipids and nucleic acid and lead to ven mutation

(Halliwell and Gutteridge, 1999). Lipid peroxidation, another possible source of ROS and other radicals, is a natural metabolic process under normal aerobic conditions. In addition, it is one of the most scrutinized results of ROS action on membrane structure and function. (Blokhina *et al.,* 2003). Higher accumulation of H_2O_2 and increased lipid peroxidation under anaerobic conditions has been reported by several researchers (Hasanuzzaman *et al.,* 2012a, b; Hossain *et al.,* 2009; Kumutha *et al.,* 2009; Sairam *et al.,* 2011). Impotence of the scavenging system to metabolize the toxic active oxygen due to either increased ROS formation or decreased activity of the scavenging enzymes is the major cause for oxidative stress (Yordanova *et al.*, 2004).

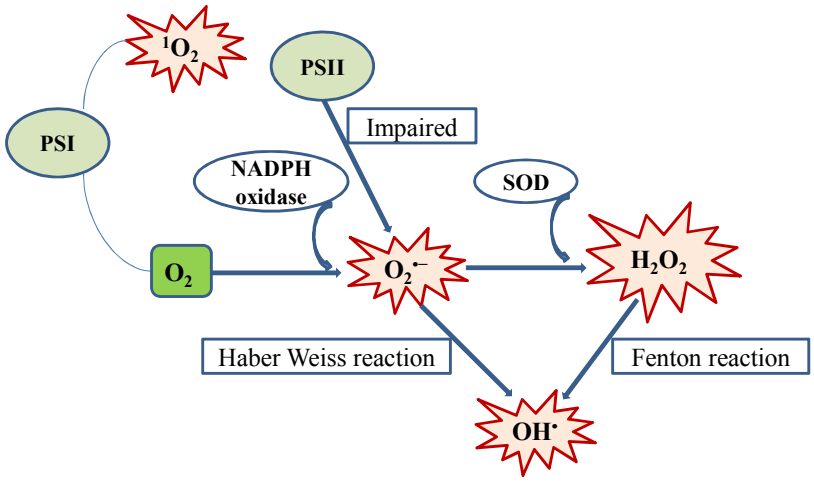

Figure 1. Production of ROS ($^1O_2$, $O_2^{\bullet-}$, $OH^{\bullet}$ and H_2O_2) under flood/waterlogging stress (Source: Hasanuzzaman *et al.*, 2017a)

However, plants have developed an endogenous antioxidant system which includes enzymatic and non-enzymatic antioxidant components to negate the toxicity of ROS, when subjected to stress. Enzymatic antioxidants are superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), glutathione *S*-transferase (GST), glutathione peroxidase (GPX) and peroxidases

(POX). And the non-enzymatic compounds such as ascorbate (AsA), glutathione (GSH), carotenoids and tocopherols and Pro (Apel and Hirt, 2004; Hasanuzzaman *et al.,* 2012a).

Table 1. Different types of oxidative stress responses by different crops under waterlogging

Crop Species	Duration of waterlogging	Oxidative stress	Reference
Citrus sinensis	45 days (continuous)	Increased SOD, CAT, APX, GR, AsA, GSH activity	Arbona *et al.* (2008)
Cajanus cajan	Upto 6 days	Increase SOD, APX, GR and CAT activity	Sairam *et al.* (2009)
Vigna angularis	Upto 6 hours	Decreased osmotic concentration of cell sap, ATP and increased the pH of theapoplastic solution	Ooume *et al.* (2009)
Chrysanthemum morifolium	Upto 8 days	Increased activity of lactate dehydrogenase (LDH), alcohol dehydrogenase (ADH), pyruvate decarboxylase (PDC), APX, CAT, SOD and ethylene production	Yin *et al.* (2009)
Oryza sativa	Upto 12 days	Increased SOD activity and fluctuated activity of CAT, APX and GR	Damanik *et al.* (2010)
Zea mays	Upto 10 days	Increased SOD, POD, CAT, APX, GR activity	Bin *et al.* (2010)
Vigna radiata	Upto 8 days	Increased SOD, APX and GR activity	Sairam *et al.* (2011)
Glycine max	Upto 3 days	Downregulation of ion transport-related proteins and upregulation of proteins involved in cytoskeletal reorganization, cell expansion, and programmed cell death	Salavati *et al.* (2012)
Sesamum indicum	Upto 8 days	Increased activity of LDH, ADH, PDC, SOD, APX and MDA upto 6 days and then mostly declined	Wei *et al.* (2013)

2.5.2 Antioxidant defense system and its role in waterlogging tolerance

When plants are exposed to stress conditions, oxidative stress results from a deficiency in ROS scavenging, either due to a surplus of ROS and/or to a decrease in the activity of the scavenging enzymes (Yordanova *et al.,* 2004; Yiu *et al.,* 2009; Wei *et al.,* 2013). But almost all plants can withdraw the harmful effects of ROS through the generation of different types of antioxidants (Ashraf, 2012). Antioxidants are of two types: enzymatic and non-enzymatic. Enzymatic antioxidants include SOD, POD, CAT, APX, MDHAR, DHAR, GR, GPX, GST etc. whereas; AsA, GHS, tocopherols and carotenoids etc. are included in non-enzymatic antioxidants (Hasanuzzaman *et al*., 2012a). There are a number of studies that revealed the production of these enzymatic and non-enzymatic antioxidants under different types and levels of stress. As a result of an insufficient supply of oxygen, root respiration is inhibited in plants exposed to waterlogging (Das *et al.*, 2009). And this leads to the production of LDH, ADH and PDC (enzymes involved in fermentative glycolysis) which showed differential activities in susceptible and tolerant varieties (Yin *et al.,* 2009; Wei *et al.,* 2013). But such fermentative metabolism is probably a temporary adaptation which allows glycolysis to continue under anoxic conditions, such as occurs during normal condition (Tadege *et al.,* 1999; Kato-Noguchi, 2002; Kato-Noguchi and Morokuma, 2007; Maricle *et al.*, 2006) as it is not able to satisfy the full demand for ATP (Gibbs and Greenway, 2003). The waste products (lactate and ethanol) also disturb cellular metabolism when allowed to accumulate (Vodnika *et al.*, 2009). According to Sairam *et al.* (2011), mung bean plants under waterlogging stress resulted an increase in the activities of various enzymatic antioxidants such as GR, SOD and APX but, Ahmed *et al.,* (2002) found reduced activities of SOD, GR, CAT and APX in the same crop. Similarly, increase in the activities of different enzymatic antioxidants was recorded

in maize seedlings when subjected to varying degree of waterlogging stress (Bin *et al.*, 2010). When pigeon pea genotypes were exposed to waterlogging stress, the activities of SOD, CAT, POD and APX also increased markedly (Sairam *et al.*, 2009). Citrus leaves showed a significant increase in total ascorbic acid, AsA, DHA, and AsA/DHA ratio in stressed plants than in control conditions when exposed to waterlogging (Arbona *et al.,* 2008). In rice seedlings, the tolerance to submergence stress can be induced by increasing the capacity of the antioxidative system (Damanik *et al.,* 2010). Higher activities of antioxidant enzymes (SOD, CAT, APX, and GR) in waterlogging tolerant varieties after 8 days of complete submergence were observed by them. Similar results were found by Bin *et al.* (2010) in maize seedlings. These studies amply clearly that when plants are exposed to waterlogged condition, the antioxidant defense system employs to scavenge the damages of oxidative stress induced by ROS.

2.6 Effect of waterlogging on sesame plants

2.6.1 Effect on growth of sesame

Sesame is marked to have the potential to sustain in drought condition and it is considered as a promising crop to be cultivated in areas with low availability of water. Though it shows drought tolerance, on the contrary it is very sensitive to excess water and wet land.

An experiment was conducted by Mensah *et al.* (2006) to evaluate the effect of simulated drought and waterlogging in sesame growth and yield. To investigate waterlogging effects in particular, they selected two treatments- flooding at 15-day-old plants (FL-1) and flooding at 30-day-old plant (FL-2). Data was measured in 50-day-old plants which showed 55 and 39 % reduction of plant height in FL-1 and FL-2

treatments compared to the plants with regular watering, respectively. They also recorded decreased number of leaves plant^{-1}, leaf area and shoot dry weight etc. in both treatments with FL-1 showing the higher reduction in all cases.

Decreased number of primary branch root, reduced root length and plant height were also reported in sesame when exposed to waterlogging condition for up to 8 days (Wei *et al.,* 2013). They used two types of sesame genotypes- ZZM2541 (tolerant) and Ezhi-2 (intolerant); to investigate the difference of their responses to waterlogging stress for different durations (0, 2, 4, 6 and 8 days). All the growth parameters they measured showed decreasing trend compared to control except primary branch root number. For the tolerant genotype it was higher than the control though in Ezhi-2 it was lower compared to the control plant.

Saha *et al.* (2016) carried out an experiment with four moderately waterlogged tolerant sesame genotypes (viz., BD-6980, BD-6985, BD-6992 and BD-7012) which were exposed to waterlogged condition for 3 consecutive days at 29 DAE. All the genotypes showed decreased plant height, leaf area and root dry when waterlogged. Root length and root volume also reduced in waterlogged condition except for BD-6992 genotype. SPAD value was recorded to increase in all waterlogged plants compared to the control plants.

Two varieties- BARI Til-2 and BARI Til-3 were used as test crop to demonstrate the effect of waterlogging stress at different durations (Sarkar *et al.,* 2016). Four treatments: Normal drainage (T1); Continuous water logging up to 12 hours (T2), 24 hours (T3) and 36 hours (T4) were selected and both the varieties were recorded to show decreasing plant height and branches plant^{-1} with the increasing duration of

waterlogging. Waterlogging could not affect the population density of these sesame varieties.

2.6.2 Effect on physiology and metabolism of sesame plant

An experiment was carried out by Xu *et al.* (2012) with three sesame genotypes: WTG-2541, WTG-2413 and WSG-EZhi2 which were exposed to flooding stress at three leaf stages for continuous 48 hrs. The result showed the net photosynthesis rate was lowest in WSG-EZhi2 (decreased by 98.32%); and WTG-2541 had the second smallest decrease (59.23%) and WTG-2413 had the least decrease (55.15%). They also measured proline content which is a marker of plant resistance to osmotic stress. Under flooding stress, proline contents of leaves increased by 72.19, 80.96 and 22.37% for WTG-2541, WTG-2413 and WSG-EZhi2, respectively.

Wei *et al.* (2013) measured chlorophyll content of leaf and soluble protein content of both leaf and root in two sesame cultivars differing in their tolerance to waterlogging stress. Both the intolerant (Ezhi-2) and tolerant (ZZM2541) cultivars showed lower chlorophyll content compared to their respective control, but the decrease was much higher in the intolerant one. Notably, in both cases chl content initially increased up to 2 days of waterlogging and then declined with increasing duration of waterlogging. Similar trend was observed in case of soluble protein content of leaves, but in root it decreased with increased duration of waterlogging. They also measured the alcohol fermentation enzymes (ADH and PDC) and lactate fermentation enzyme (LDH) in sesame roots which denotes the fermentative metabolism of roots under waterlogging stress. The activity of ADH increased up to 6 days of waterlogging and then dropped in case of both cultivars. Same trend of increasing was observed in LDH activity of the intolerant cultivar but for tolerant cultivar it was peak after 2 days of

waterlogging. In case of PDC, the tolerant cultivar showed highest activity after 4 days of waterlogging and then declined gradually, but the intolerant one showed erratic pattern of increase and decrease with a peak after 2 days of waterlogging.

2.6.3 Effect on sesame yield

Mensah *et al.* (2006) reported that with the increased duration of flooding the yield attributes like pods plant^{-1}, seeds plant^{-1} and yield plant^{-1} are reduced. Yield plant^{-1} was decreased by 73% in plants flooded at the age of 15 days and 17% in plants flooded at the age of 30 days. However, they showed flooding has no effect on 1000 seed weight of sesame.

An experiment was conducted by Sun *et al.* (2009) to investigate the effect of waterlogging on the sub-stages of flowering stage: budding, full flowering and ending of flowering stages. Sesame plants were waterlogged for up to 0, 24, 36, 48 and 60 hours and the full flowering and budding stage were recorded to be more susceptible than ending of flowering stage with higher withering and death rate. Yield parameters i.e., number of capsule plant^{-1}, number of seed plant^{-1} and yield plant^{-1} were also lower in plants facing flood during these two stages. However, irrespective of the flowering stages, all the plants exposed to waterlogging stage showed decrease yield attributes with the increasing duration of waterlogging.

In order to explore the effect of duration of waterlogging on yield of sesame, Sarkar *et al.* (2016) performed an experiment with two sesame varieties and reported that pods plant^{-1}, 1000 seed weight and yield (kg ha^{-1}) are reduced with increased waterlogging periods. After 12, 24 and 36 hrs of waterlogging, yield reduction of BARI til 2 was recorded as 24, 38 and 39.41% of control, and in BARI til 3 it was 29, 46 and 53% of control, respectively.

Similarly, decreased capsule plant^{-1}, seed capsule^{-1} and seed weight plant^{-1} were recorded by Saha *et al.* (2016) in four sesame genotypes. However, they found very little effect of waterlogging treatment (3 d) on the 1000-seed weight of these genotype, except one (BD 6992) showing a decrease by 7%, compared to control.

2.6.4 Oxidative stress in waterlogged sesame

Xu *et al.* (2012) tested three sesame genotypes for 48 hrs of waterlogging and measured the MDA content in leaves after completion of stress duration. MDA contents of leaves in three genotypes- WTG-2541, WTG 2413 and WSG-EZhi2 were increased by 7.74, 2.10 and 28.73%, respectively denoting that the later one is more susceptible to waterlogging and the 2nd one is the most tolerant one among these three genotypes.

In the experiment conducted by Wei *et al.* (2013), they measured MDA content in two different cultivars one of which is tolerant (ZZM2541) to waterlogging and another intolerant (Ezhi-2). It was much higher in the waterlogged Ezhi-2 plants than in the non-waterlogged ones throughout the waterlogging period, increasing by about 1.3-fold (day 2) and 1.8-fold by day 6, and subsequently declining. However, the level in the intolerant one was over 1.6-fold higher than that in the tolerant one on the 8th day. In ZZM2541, MDA content was notably unaffected by waterlogging stress.

Saha *et al.* (2016) also measured MDA contents of four waterlogged sesame genotypes, which were under stress for 3 days. Among the four genotypes BD 6980 showed the lowest increase (5.79%) in MDA and BD 1012 showed the highest (48.2%) increase of MDA content in sesame under waterlogged condition.

2.6.5 Antioxidant enzyme activities of waterlogged sesame

Sun *et al.* (2009) measured three enzyme activities namely POD, SOD and CAT to evaluate the effect of waterlogging on leaf protective enzymes of sesame. The data showed that POD activity increased up to 36 hrs of waterlogging and later on decreased up to 60 hrs of waterlogging. But, in case of SOD and CAT activities, they both increased up to 24 hrs of waterlogging and then the activities dropped. Comparing among the flowering stages, POD and CAT activities were higher in full flowering stage, and SOD activity was higher in budding stages when subjected to waterlogging condition.

Similarly, Xu *et al.* (2012) also measured the POD, SOD and CAT activities in three sesame genotypes after 48 hrs of waterlogging treatment and found increased SOD activity in all three genotypes. On the other hand, CAT activity decreased under waterlogging stress and POD activity decreased only in one genotype- WSG-EZhi2 while in other two-WTG-2541 WTG-2413, it was higher compared to control.

Ascorbate peroxidase (APX) activities was measured in two sesame cultivars namely ZZM2541 (tolerant) and Ezhi-2 (intolerant) under different duration of waterlogging up to 8 days (Wei *et al.,* 2013). In Ezhi-2, it slightly increased up to the 4^{th} day and then slightly dropped, but in ZZM2541 it was about 8-folds higher only after 2 days of waterlogging and 9-folds higher on 6^{th} day. They also measured the SOD and CAT activities, both of which increased at 8^{th} day of waterlogging. However, CAT activity was highest (2.3-times higher) in the tolerant cultivar on 2^{nd} day of waterlogging.

Four sesame genotypes were tested after 3 days of waterlogging for the measurement of different enzyme activities: POD, CAT, APX, GPX and SOD by Saha *et al.* (2016). Among these CAT, APX and GPX activities increased in all genotypes. But, POD

activity decreased in BD 7012 genotype while increased in other three. And SOD activity decreased BD 7012 and BD 6992 genotypes with an increase of 10.63 and 32.75% in BD 6980 and BD 6985, respectively. However, BD 6980 showed the highest APX (163.19%) and GPX (157.66%) activities, and BD 6985 showed the highest (104.55%) CAT activities compared to their respective control.

From the above explained review it was evident that waterlogging as an abiotic stress has harmful effects on plant growth, physiology and productivity. Waterlogging creates oxidative stress in crops including sesame which leads to production of highly reactive ROS which in higher amount leads to cell death. It was also demonstrated that plants possess some adaptive measures and antioxidant defense system which may possibly lead to plant survival. However, very few studies were documented regarding waterlogging induced oxidative stress and activation of antioxidant defense system in sesame plants. Therefore, a higher scope of further research on this aspect should be availed worldwide.

Chapter 3

MATERIALS AND METHODS

This chapter represents a concise description about the experimental time, site, climatic condition, seed or planting materials, treatments, experimental design and layout, crop growing procedure, fertilizer application, intercultural operations, data collection and statistical analysis of the experiment.

3.1 Location

The experiment was conducted in two locations:

i. The field experiment (Experiment-1) to study the morpho-physiological and yield attributes was conducted at the experimental shed of the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka (90º 77′ E longitude and 23º 77′ N latitude), Bangladesh, during the period from June 2015 to August 2015. The location of the experimental site has been shown in Appendix I.

ii. The greenhouse experiment (Experiment-2) to study the oxidative stress responses was conducted in the greenhouse of Faculty of Agriculture, Kagawa University, Kagawa (134° 07′ 60.00′′ E longitude and 34° 15′ 60.00′′ N latitude), Japan, during the period of June 2016 to August 2016.

3.2 Characteristics of Soil

The soil of experiment-1 belonged to the Modhupur tract (AEZ No. 28). It was a medium high land with non-calcarious dark grey soil. The pH value of the soil was 5.6. The physical and chemical properties of the experimental soil have been shown in Appendix II.

The soil of experiment-2 was of same pH and similar physical and chemical properties.

3.3 Weather condition of experimental site

The area of experiment-1 was under the subtropical climate and was characterized by high temperature, high humidity and heavy precipitation with occasional gusty winds during the period from April to September. The detailed meteorological data in respect of air temperature, relative humidity, rainfall and sunshine hour recorded by the meteorology center, Dhaka for the period of experimentation have been presented in Appendix III.

The area of experiment-2 was a greenhouse situated inside the Faculty of Agriculture, Kagawa University, Kagawa, Japan with an average temperature of 35ºC and 60% relative humidity (RH) maintained.

3.4 Materials

3.4.1 Plant materials

Only one sesame variety BARI Til-4 was used in both experiments. This variety was released in 2009. Plant height is 90-120 cm, seeds are deep brown bold and flower color is white. About 70% pods are eight chambered. Crop Duration is 90-95 days with a yield of 1.25-1.5 t ha^{-1}.

3.4.2 Plastic pot

Empty plastic pots with 18 inch depth and 14 inch diameter were used for both experiments. Twelve kilograms sun-dried soils along with organic manures and fertilizers were put in each pot. After that, pots were prepared for seed sowing.

3.5 Treatments

Experiment-1 consisted of the following treatments:

i. Control (well-drained)
ii. Waterlogged for 2 days at vegetative stage
iii. Waterlogged for 4 days at vegetative stage
iv. Waterlogged for 6 days at vegetative stage
v. Waterlogged for 2 days at reproductive stage
vi. Waterlogged for 4 days at reproductive stage
vii. Waterlogged for 6 days at reproductive stage
viii. Waterlogged for 2 days at maturity stage
ix. Waterlogged for 4 days at maturity stage
x. Waterlogged for 6 days at maturity stage

Treatments were started at 21 DAS for vegetative stage, 35 DAS for reproductive stage and 55 DAS for maturity stage.

Experiment-2 consisted of the following treatments:

i. Control (well-drained)
ii. Waterlogged for 2 days
iii. Waterlogged for 4 days
iv. Waterlogged for 6 days
v. Waterlogged for 8 days

Treatment was given at vegetative stage (21 DAS).

3.6 Design and layout of the experiment

Both the experiments were laid out in a Randomized Completely Block Design (RCBD) with three replications.

In experiment-1 there were two sets of pot- 1st set with 36 pots for measuring growth parameters with one control for each stage; and 2nd set with 30 pots for measuring yield parameters.

In experiment-2 there were 24 pots with one control for each treatment.

3.7 Seed collection

Seeds of BARI Til 4 were collected from Oil Crop Research Centre, Bangladesh Agriculture Research Institute (BARI), Joydebpur, Gazipur. Seeds from same lot were used in both experiments.

3.8 Pot preparation

Pot preparation was same for both experiments. The collected soil was sun dried, crushed and sieved. The soil, organic manure and fertilizers were mixed well before placing the soils in the pots. Soils of the pots were poured in polythene bag. Each pot was filled up with 12 kg soil. For experiment-1 pots were placed at the net house of Sher-e Bangla Agricultural University, Bangladesh and experiment-2 at the greenhouse of Faculty of Agriculture, Kagawa University, Japan. The pots were pre-labeled for each variety and treatment. Finally, water was added to bring soil water level to field capacity.

3.9 Fertilizer application

Fertilizers used in the experimental pots were organic manure, urea, triple super phosphate, muriate of potash and gypsum at the rate given value in a tabulated form. The whole amount of fertilizers was incorporated with soil at final pot preparation before sowing.

Fertilizer doses are as follows:

Fertilizers	Dose (kg ha^{-1})	Actual amount/pot (g)
Organic manure	-	3 kg
Urea	120	2.5
Triple super phosphate	140	3.2
Muriate of potash	40	1.0
Gypsum	100	2.4

3.10 Seed sowing technique

Thirty healthy seeds were sown in each pot. After germination 5 plants were allowed to grow in each pot.

3.11 Intercultural operations

3.11.1 Gap filling and thinning

After sowing seeds continuous observation was kept. It was observed that no single seed failed to germinate. So, there was need of gap filling. Keen observation was made for thinning to maintain 5 seedlings. Thinning was done to maintain spacing of the plants.

3.11.2 Weeding and irrigation

Sometimes there were some weeds observed in pots which were uprooted manually. Irrigation was given to maintain field capacity moisture level.

3.11.3 Plant protection measure

There was no insect pests appeared. Moreover, the pots were protected by netting to prevent birds.

3.12 General observation of the experimental pots

Observations were made regularly and the plants looked normal green. The maximum flowering stage and pod initiation were not uniform.

3.13 Collection of data

Growth and physiological parameters of experiment-1 were collected after the completion of treatment duration at every stage. The yield parameters were recorded at harvest. Biochemical parameters of experiment-2 were collected after the completion of treatment durations every time.

Data were collected on following parameters:

3.13.1 Crop growth parameters:

- Mortality rate
- Plant height
- Number of leaves plant^{-1}
- Leaf area plant^{-1}
- Above ground fresh weight plant^{-1}
- Above ground dry matter weight plant^{-1}

3.13.2 Physiological parameters:

- SPAD value of leaf
- Relative water content (RWC)
- Chlorophyll *a*, *b* and carotenoid contents

3.13.3 Anatomical pictures

Pictures of stem transverse sections were taken with the help of digital microscope.

3.13.4 Oxidative stress indicators:

- Lipid peroxidation
- H_2O_2 content
- Proline content
- Methylglyoxal content
- Ascorbic acid content
- Glutathione content
- Activities of antioxidant enzymes (CAT, APX, MDHAR, DHAR, GR, GPX, Gly I and Gly II)

3.13.5 Yield contributing parameters:

- Plant height at yield
- Capsule plant^{-1}
- Seed capsule^{-1}
- 1000-seeds weight
- Grain yield plant^{-1}
- Stover yield plant^{-1}
- Biological yield plant^{-1}
- Harvest index

3.14 Procedure of sampling for growth study during the crop growth period

3.14.1 Mortality rate

Total number of plants per pot was counted before starting the treatment which may be denoted as Ni and total number of plants per pot was counted again after the completion of treatment duration which may be denoted as Np. Mortality rate was calculated using the following formula:

$$\text{Mortality rate (\%)} = \frac{\text{Ni}-\text{Np}}{\text{Ni}} \times 100$$

3.14.2 Plant height

The height of the sesame plants was recorded in plants after the duration of treatment was completed, beginning from the ground level up to tip of the leaf was counted as height of the plant. The average height of five plants was considered as the height of the plant for each pot.

3.14.3 Number of leaves plant^{-1}

The leaves of each plant were counted after the treatment duration completed. Average number of leaves of five plants was considered as the total leaves plant^{-1}.

3.14.4 Leaf area plant^{-1}

The leaf area plant^{-1} was determined according to Pereira *et al.* (2014) by measuring the length (in cm) of 5 leaves, and counting the total number of leaves per plant, applying the following equation, $S = 0.3552 \times C^2$, where S = leaf area (cm^2) and C = leaf length (cm) and then multiplying the leaf area by the total number of leaves per plant to obtain the total leaf area plant^{-1} (cm^2).

3.14.5 Fresh weight plant^{-1}

Three sample plants uprooted from each pot randomly and washed them in water. Then the plants were weighed in a balance and averaged them to have fresh weight plant^{-1} and taken after completion of treatment duration.

3.14.6 Dry weight plant^{-1}

Three sample plants after weighing for fresh weight was dried them in an electric oven maintaining 60 °C for 48 hours. Then the plans were weighed in an electric balance and averaged them to have dry weight plant^{-1}. The data were collected after completion of treatment duration.

3.15 Procedure of sampling physiological parameters

3.15.1 SPAD value

Three leaves were randomly selected from each pot. The top and bottom of each leaflet were measured with atLEAF (FT Green LLC, USA) as atLEAF value. Then it was averaged and total chlorophyll content was measured by the conversion of atLEAF value into SPAD units and then total chl content was measured.

3.15.2 Relative water content

Relative water content (RWC) was measured according to Barrs and Weatherly (1962). Whole leaf discs were weighed as FW and then floated on distilled water in Petri dishes and kept in a dark place. After 8 h, the leaf discs were weighed again after removing excess surface water, and considered as turgid weight (TW). Finally, DW was measured after drying at 80 °C for 48 h. Leaf RWC was calculated using the following formula:

$$\text{RWC (\%)} = \frac{FW-DW}{TW-DW} \times 100$$

3.15.3 Photosynthetic pigments

Photosynthetic pigments were measured following the method of Arnon (1949). The leaves (0.5 g) were extracted with 10 mL 80% v/v acetone and supernatant was obtained by centrifuging at 2000 × g for 10 min. After diluting the supernatant, the absorbance was measured with a UV-visible spectrophotometer at 663, 645 and 480 nm for chl *a*, chl *b* and carotenoid contents, respectively.

3.16 Procedure of observing anatomical responses

At the end of each waterlogging treatment, samples were taken from the stem (3 cm above the flood level), transversely sectioned into thin segments, double stained with Safranine mixture, mounted in glycerin and photographed with a tetra view LCD microscope (Celestron, USA).

3.17 Procedure of measuring oxidative stress indicators

3.17.1 Measurement of lipid peroxidation

The level of lipid peroxidation was measured by estimating malondialdehyde (MDA) content according to Heath and Packer (1968) with slight modification by Hasanuzzaman *et al.* (2012b). Leaf samples (0.5 g) were homogenized in 3 mL 5% (w/v) trichloroacetic acid (TCA), and the homogenate was centrifuged at 11,500 × g for 15 min. The supernatant (1 mL) was mixed with 4 mL of thiobarbituric acid (TBA) reagent (0.5% of TBA in 20% TCA). The reaction mixture was heated at 95 °C for 30 min in a water bath and then quickly cooled in an ice bath and centrifuged again at 11,500 × g for 10 min. The absorbance of the colored supernatant was measured at 532 nm and was corrected for non-specific absorbance at 600 nm. MDA

content was calculated by using extinction coefficient 155 mM^{-1} cm^{-1} and expressed as nmol g^{-1} FW.

3.17.2 Determination of hydrogen peroxide (H_2O_2) content

Hydrogen peroxide (H_2O_2) was determined according to the method of Yu *et al.* (2003). Leaf tissue (0.5 g) was homogenized with 3 mL of 50 mM potassium–phosphate (K–P) buffer (pH 6.5) at 4 °C. The homogenate was centrifuged at 11,500 × g for 15 min. The supernatant (2 mL) was mixed with 666.4 μL of 0.1% $TiCl_4$ in 20% H_2SO_4 (v/v) and was kept at room temperature for 10 min. After that, the mixture was again centrifuged at 11,500 × g for 12 min. The supernatant was then measured spectrophotometrically at 410 nm to determine H_2O_2 content using extinction coefficient 0.28 μM^{-1} cm^{-1} and was expressed as nmol g^{-1} FW.

3.17.3 Measurement of methylglyoxal level

Methylglyoxal was measured following the method of Wild *et al.* (2012). Leaves were homogenized in 5% perchloric acid and centrifuged at 4 °C for 10 min at 11,000 ×g. The supernatant was decolorized by adding charcoal. The decolorized supernatant was neutralized by adding a saturated solution of sodium carbonate at room temperature. The neutralized supernatant was used to estimate MG by adding sodium dihydrogen phosphate and N-acetyl-L-cysteine to a final volume of 1 mL. Formation of the product N-α-acetyl-*S*-(1-hydroxy-2-oxoprop-1-yl) cysteine was recorded after 10 min at a wavelength of 288 nm, and the MG content was calculated using a standard curve of known concentration.

3.17.4 Extraction and measurement of ascorbate and glutathione

Fresh leaves (0.5 g) were homogenized in 3 mL ice-cold 5% meta-phosphoric acid containing 1 mM ethylenediaminetetraacetic acid (EDTA) using a mortar and pestle. The homogenate was centrifuged at 11,500 × g for 12 min at 4 °C, and the supernatant was collected to analyze for AsA and GSH. Ascorbate content was determined following the method of Huang *et al.* (2005) with some modifications. The supernatant was neutralized with 0.5 M K-P buffer (pH 7.0), and the oxidized fraction was reduced by 0.1 M dithiothretitol. AsA was assayed spectrophotometrically at 265 nm in 100 mM K-P buffer (pH 7.0) with 0.5 units of ascorbate oxidase (AO). A specific standard curve of AsA was used for quantification. The GSH pool was assayed according to a previously described method (Yu *et al*. 2003) with modifications as described by Paradiso *et al.* (2008). Aliquots (0.2 mL) of supernatant were neutralized with 0.3 mL of 0.5 M K-P buffer (pH 7.0). Based on enzymatic recycling, GSH is oxidized by 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB) and reduced by nicotinamide adenine dinucleotide phosphate (NADPH) in the presence of GR, and GSH content was evaluated by the rate of absorption changes at 412 nm of 2-nitro-5-thiobenzoic acid (NTB) generated from the reduction of DTNB. Oxidized glutathione (GSSG) was determined after removing GSH by 2-vinylpyridine derivatization. Standard curves with known concentrations of GSH and GSSG were used. The content of GSH was calculated by subtracting GSSG from total GSH.

3.17. 5 Measurement of proline content

Proline content was measured according to Bates *et al.* (1973). Fresh leaf tissue (0.5 g) was homogenized in 5 mL of 3% sulfosalicylic acid in an ice-cold condition, and

the homogenate was centrifuged at 11,500 ×g for 15 min. The supernatant (1 mL) was mixed with 1 mL of acid ninhydrin and 1 mL of glacial acetic acid, and the mixture was placed in a water bath (100 ℃) for 1 h. The mixture was then transferred to a test tube and kept on ice for cooling. Toluene (2 mL) was added to the cooled mixture and mixed thoroughly using a vortex machine. After few minutes, chromophore-containing toluene was read spectrophotometrically at 520 nm. The proline content of the sample was determined by comparing with a standard curve of known concentration of Pro.

3.17.6 Enzyme extraction and assays

Leaf tissue (0.5 g) was homogenized in 1 mL of 50 mM icecold K-P buffer (pH 7.0) containing 100 mM KCl, 1 mM AsA, 5 mM β-mercaptoethanol, and 10% (w/v) glycerol using a pre-cooled mortar and pestle. The homogenates were centrifuged at 11,500 ×g for 10 min and the supernatants were used to determine enzyme activity. A temperature of 0–4 ℃ was maintained for all the activities.

Catalase (CAT; EC: 1.11.1.6) activity was assayed following the method of Hasanuzzaman *et al.* (2012b) by monitoring the decrease in absorbance at 240 nm for 1 min caused by the decomposition of H_2O_2. The reaction mixture contained 50 mM K-P buffer (pH 7.0), 15 mM H_2O_2, and enzyme solution in a final volume of 700 µL. The reaction was initiated with the enzyme extract and activity was calculated using extinction coefficient 39.4 M^{-1} cm^{-1}.

Ascorbate peroxidase (APX; EC: 1.11.1.11) activity was determined according to the method of Nakano and Asada (1981) with a solution mixture of 50 mM K-P buffer (pH 7.0), 0.5 mM AsA, 0.1 mM H_2O_2, 0.1 mM EDTA, and enzyme extract in a final

volume of 700 µL. The activity was measured by observing the decrease in absorbance at 290 nm for 1 min using extinction coefficient 2.8 mM^{-1} cm^{-1}.

Monodehydroascorbate reductase (MDHAR, EC: 1.6.5.4) activity was measured following the method described in Hossain *et al.* (1984). The reaction mixture contained 50 mM Tris-HCl buffer (pH 7.5), 0.2 mM NADPH, 2.5 mM AsA, 0.5 units of AO, and enzyme solution in a final volume of 700 µL. The absorbance was measured at 340 nm for 1 min and activity was calculated using extinction coefficient 6.2 mM^{-1} cm^{-1}.

Dehydroascorbate reductase (DHAR; EC: 1.8.5.1) activity was assayed according to the method of Nakano and Asada (1981). The reaction buffer contained 50 mM K-P buffer (pH 7.0), 2.5 mM glutathione (GSH), 0.1 mM EDTA, and 0.1 mM dehydroascorbate (DHA). The activity was measured from the change in absorbance at 265 nm for 1 min and calculated using extinction coefficient 14 mM^{-1} cm^{-1}.

Glutathione reductase (GR; EC: 1.6.4.2) activity was assayed according to the method of Hasanuzzaman *et al.* (2012b) by monitoring the decrease in absorbance at 340 nm for 1 min. The reaction mixture contained 0.1 M K-P buffer (pH 7.0), 1 mM EDTA, 1 mM GSSG, 0.2 mM NADPH, and enzyme extract. The activity was calculated using extinction coefficient 6.2 mM^{-1} cm^{-1}.

Glutathione peroxidase (GPX; EC: 1.11.1.9) activity was assayed using the method of Elia *et al.* (2003). The reaction mixture consisted of 100 mM K-P buffer (pH 7.0), 1 mM EDTA, 1 mM sodium azide (NaN_3), 0.12 mM NADPH, 2 mM GSH, 1 unit GR, 0.6 mM H_2O_2 (as a substrate), and 20 µL of sample solution. The oxidation of NADPH was recorded at 340 nm for 1 min and the activity was calculated using extinction coefficient 6.62 mM^{-1} cm^{-1}.

Glyoxalase I (Gly I; EC: 4.4.1.5) activity was determined following the method of Hasanuzzaman *et al.* (2012b). The assay mixture contained 100 mM K–P buffer (pH 7.0), 15 mM magnesium sulphate, 1.7 mM GSH, and 3.5 mM MG in a final volume of 700 µL. The increase in absorbance was recorded at 240 nm for 1 min. The activity was calculated using extinction coefficient 3.37 mM^{-1} cm^{-1}.

Glyoxalase II (Gly II; EC: 3.1.2.6) activity was determined according to Principato *et al.* (1987). The reaction mixture contained 100 mM Tris-HCl buffer (pH 7.2), 0.2 mM DTNB, and 1 mM *S*-D-lactoylglutathione (SLG). The change in absorbance was recorded at 412 nm, and the activity was calculated using extinction coefficient 13.6 mM^{-1} cm^{-1}.

3.18 Procedure of measuring yield and yield contributing parameter

3.18.1 Plant height

Plant height was measured from the soil level to the apex of the leaf or spike in randomly 5 plants of each pot.

3.18.2 Total number capsule plant^{-1}

The total number of capsule plant^{-1} was counted from selected ten samples and then averaged.

3.18.3 Total number of seed capsule^{-1}

Ten capsules from each pot were selected and seeds were counted from each individual capsule and then averaged.

3.18.4 1000-seed weight

One hundred clean sun dried grains were counted from the seed stock obtained from the sample plants and weighed by using an electronic balance. Then it was converted into thousand grain weight.

3.18.5 Grain yield plant^{-1}

The grains were separated by threshing per plant and then sun dried and weighed.

3.18.6 Stover yield plant^{-1}

The grains were separated by threshing and then the plants were sun dried and weighed.

3.18.7 Biological yield plant^{-1}

Biological yield was calculated by using the following formula:

Biological yield= Grain yield + straw yield

3.18.8 Harvest index

It denotes the ratio of economic yield to biological yield and was calculated following the formula of Gardner *et al.* (1985). It was calculated by using the following formula:

Harvest index (HI) = $\frac{Grain\ yield}{Biological\ yield}$x 100

3.19 Statistical analysis

The data obtained for different parameters were statistically analyzed following computer based software XLSTAT 2016 (AddinSoft, 2016) and mean separation was done by LSD at 5% level of significance.

Chapter 4

RESULTS AND DISCUSSION

Experiment-1

4.1 Crop growth parameters

4.1.1 Mortality rate

Waterlogging can lead to death of sesame plants. The death rate was higher in the earlier stages of sesame growth. In this experiment, it was observed that more plants died in the pots waterlogged during the vegetative stage, compared to the pots waterlogged during reproductive and maturity stages (Figure 2). Higher duration of waterlogging treatment also increased the number of dead seedlings in all stages of sesame growth. However, control or well-drained plants showed no mortality of seedlings which ensures the sole reason of plant death as waterlogging.

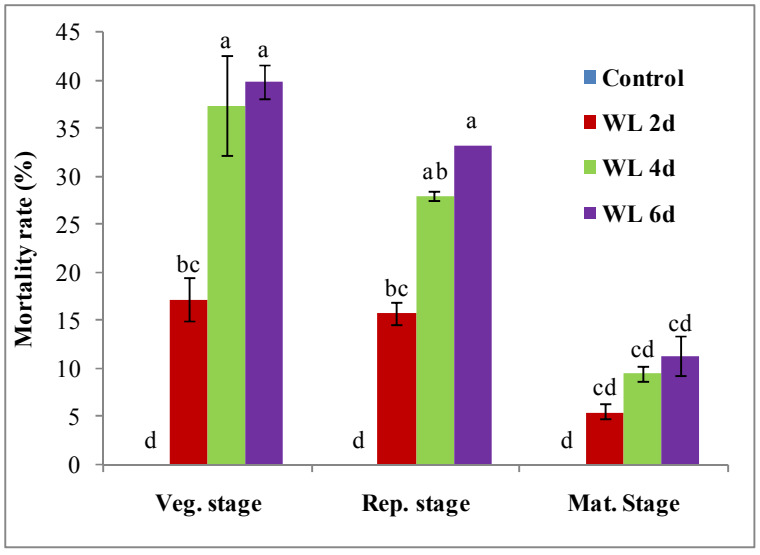

Figure 2. Effect of waterlogging stress on mortality rate of sesame seedlings. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.1.2 Plant height

Plant height of sesame plants showed no significant changes during the vegetative stage of seedling growth after waterlogging. But, it reduced significantly at reproductive stage when waterlogged for 4 and 6 days. At maturity, plant height decreased by 10 and 17% in plants waterlogged for 4 and 6 days respectively, compared to the control plants (Figure 3). Reduction in plant height of sesame plants under waterlogging or flooding stress was also reported by several other researchers (Mensah *et al*., 2006; Wei *et al*., 2013; Saha *et al*., 2016).

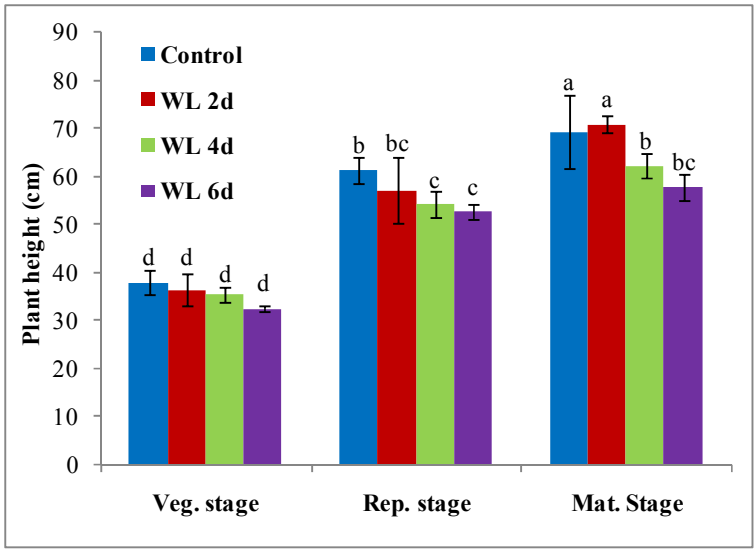

Figure 3. Effect of waterlogging stress on plant height of sesame. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.1.3 Number of leaves plant^{-1}

Significant reduction of leaves plant^{-1} has been observed in plants waterlogged either during reproductive stage or maturity stage. Waterlogging treatments during reproductive stage showed lower number of leaves plant^{-1} by 44, 55 and 59% at 2, 4 and 6 days of waterlogging respectively, compared to the same aged well-drained plants (Figure 4). Plants waterlogged for 4 and 6 days at maturity stage showed

reduced number of leaves plant^{-1}, but higher in plants waterlogged for 2 days. This may be because later stages of plant growth are less damaged with short duration stress. However, leaves were observed to become less affected by waterlogging stress during the vegetative stage (Figure 4). Prasanna and Rao (2014) reported that number of leaves plant^{-1} decreased due to waterlogging stress in green gram plants.

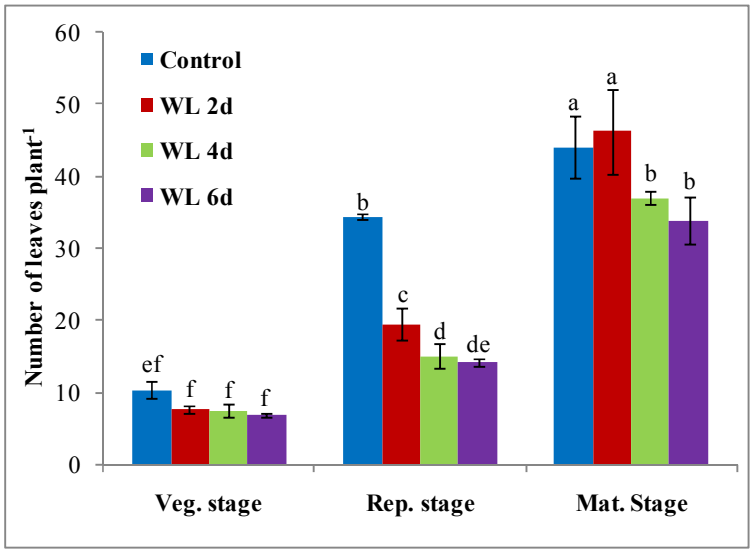

Figure 4. Effect of waterlogging stress on number of leaves plant^{-1} of sesame. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.1.4 Leaf area plant^{-1}

Though the leaf numbers were not significantly affected by waterlogging during vegetative stage, leaf area reduced remarkably in the plants waterlogged for 6 days at this stage (Figure 5). Significant reduction of leaf area under waterlogging stress has been exhibited in tobacco (Yu and Rengel, 1999), barley (Zhang *et al*., 2007), mung bean (Kumar *et al*., 2013), green gram (Prasanna and Rao, 2014) and sesame (Mensah *et al*., 2006; Saha *et al*., 2016).

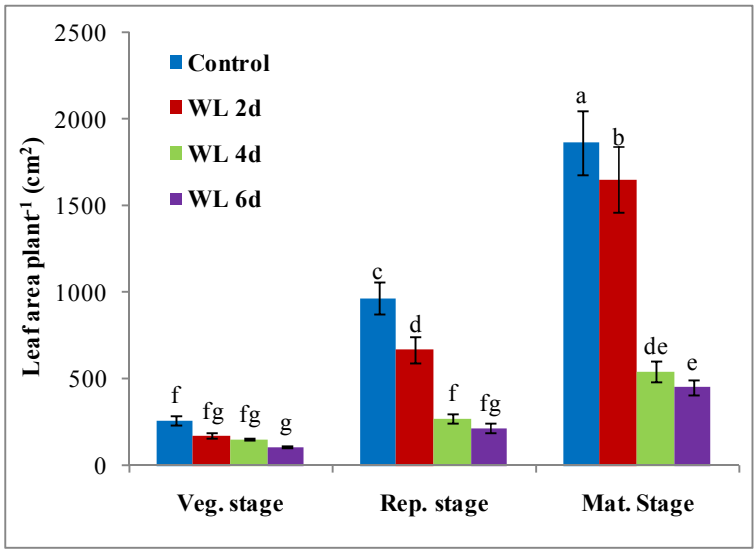

Figure 5. Effect of waterlogging stress on leaf area plant^{-1} of sesame. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

This experiment also showed significant reduction of leaf area by 30, 71 and 77% at reproductive stage and 11, 70 and 76% at maturity stage in plants waterlogged for 2, 4 and 6 days, respectively compared to the control (Figure 5).

4.1.5 Above ground fresh weight plant^{-1}

As plant height showed little changes under waterlogging condition at vegetative stage, there was also less effect on above ground fresh weight plant^{-1}, except the plants waterlogged for 6 days. But, in case of reproductive stage, FW reduced significantly in all waterlogging treatments (Figure 6). At maturity, 4 and 6 days of waterlogging duration reduced their FW which were statistically similar between these two (Figure 6). Plants waterlogged for longer duration are prone to wilting and thus reduced FW is obtained. In tobacco (Yu and Rengel, 1999) and barley (Zhang *et al*., 2007) similar results were demonstrated.

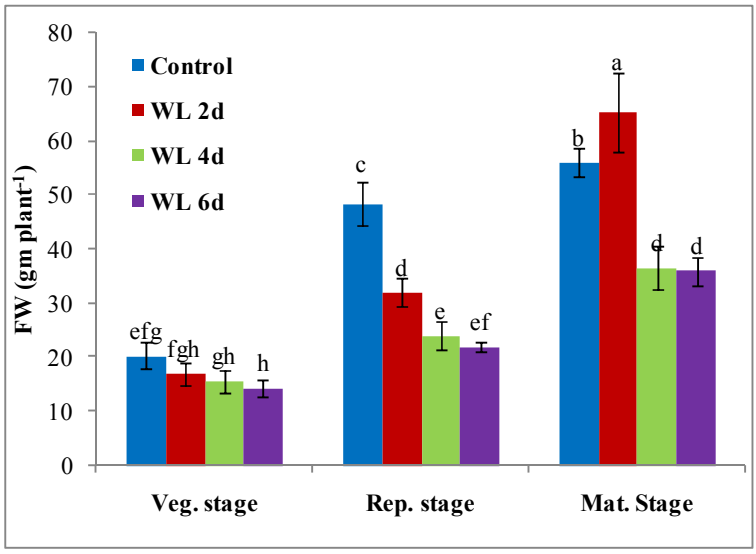

Figure 6. Effect of waterlogging stress on above ground fresh weight (FW) plant^{-1} of sesame. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.1.6 Above ground dry matter weight plant^{-1}

Plant DW showed similar results as plant FW. As shown in Figure 7, DW remarkably reduced in plants waterlogged at reproductive stage, but at vegetative stage only affected the longer duration (6 days) of waterlogging treatment. However, at maturity plants waterlogged only for 4 and 6 days showed lower DW, whereas in plants waterlogged for 2 days it was higher (Figure 7). Mensah *et al*. (2006) observed significant reduction of both shoot and root DW in sesame seedlings under prolonged flood condition.

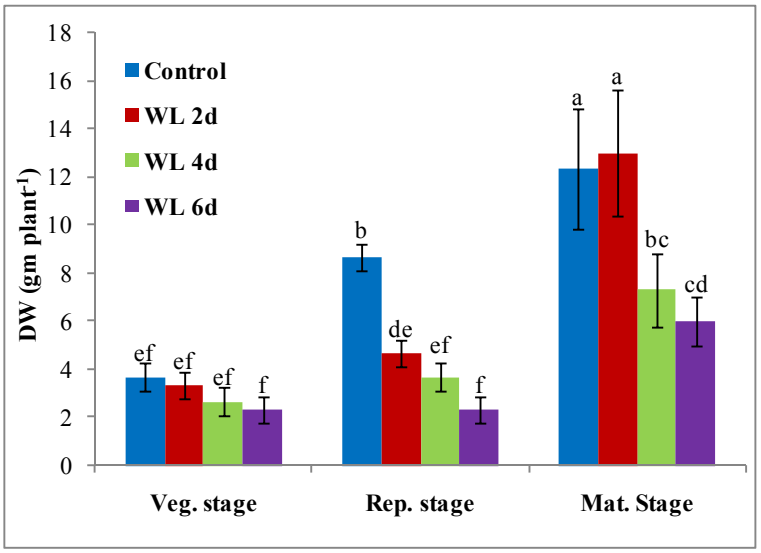

Figure 7. Effect of waterlogging stress on above ground dry weight (DW) plant^{-1} of sesame. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.2 Physiological parameters

4.2.1 SPAD value

SPAD reading which is the indicator of chl content of leaf showed lower value in the leaves of waterlogged plants compared to the control plants (Figure 8) which is supported by the result of Tan *et al*., (2008) in wheat plants. But, Saha *et al*. (2016) found opposite results (higher SPAD reading at waterlogging) may be because they used some waterlogging tolerant genotypes of sesame in their experiment.

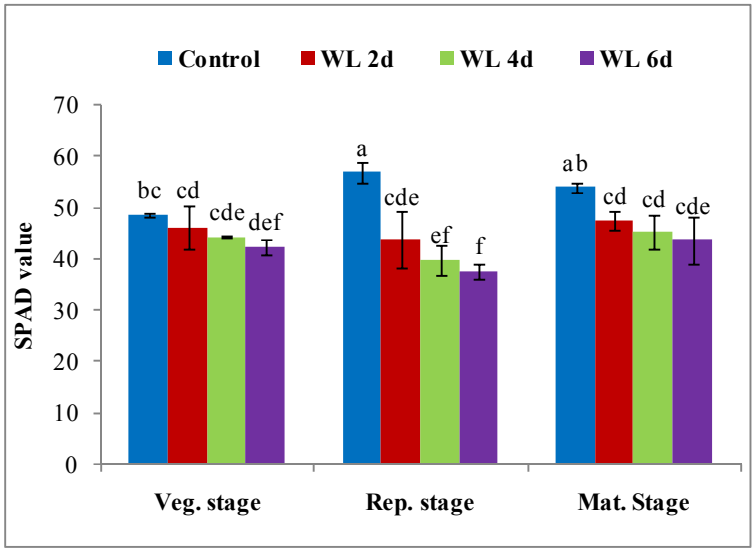

Figure 8. Effect of waterlogging stress on SPAD value of sesame. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.3 Anatomical study

The most common anatomical responses of plants in waterlogging condition are characterized as the formations of aerenchyma and adventitious roots. These responses at anatomical level enables the plants to facilitate the oxygen capture for submerged tissues, which ultimately alleviate the hypoxic conditions (Colmer, 2003; Suralata and Yamauchi, 2008; Wei *et al*., 2013). This contributes to the survival of plants in frequently waterlogged soils. In this experiment, we made the thin transverse sections of waterlogged sesame stem at the level of waterlogging. Later it was observed under digital microscope which showed the formation of some distinct aerenchyma due to waterlogging (Figure 09). Similar aerenchyma formations were studied anatomically in two *Dendranthema* species (Yin *et al*., 2010) and sesame (Wei *et al*., 2013).

Adventitious roots are mostly formed in plants showing some tolerance to waterlogging. Sesame is sensitive to waterlogging and BARI Til 4 is also considered as a susceptible one. However, the anatomical study of our experiment observed some tiny adventitious root initiating at the water level of waterlogged sesame plants as shown in Figure 10.

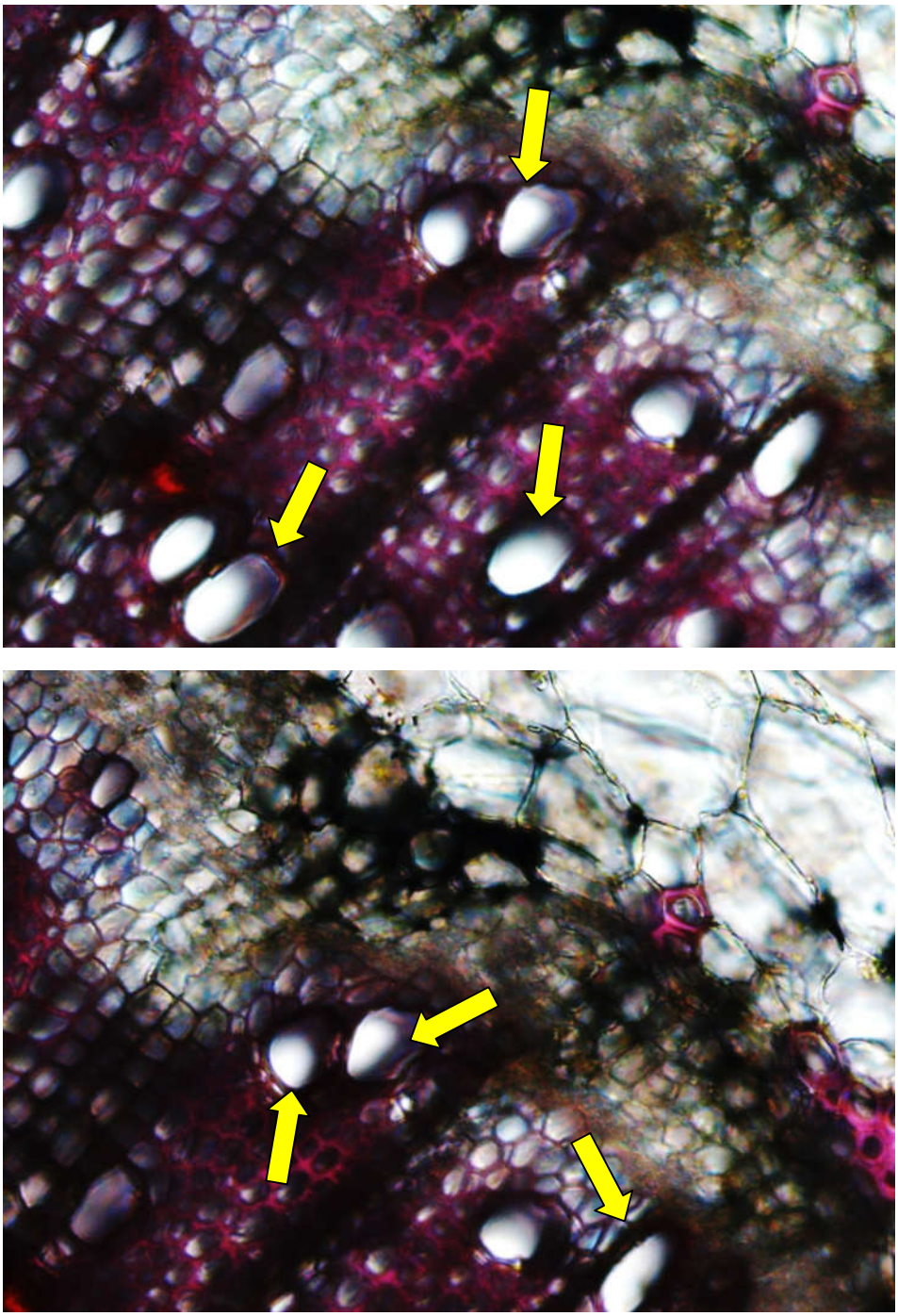

Figure 09. Transverse stem sections of waterlogged sesame plants showing aerenchyma formation. Arrows in yellow indicates lysigenous aerenchyma.

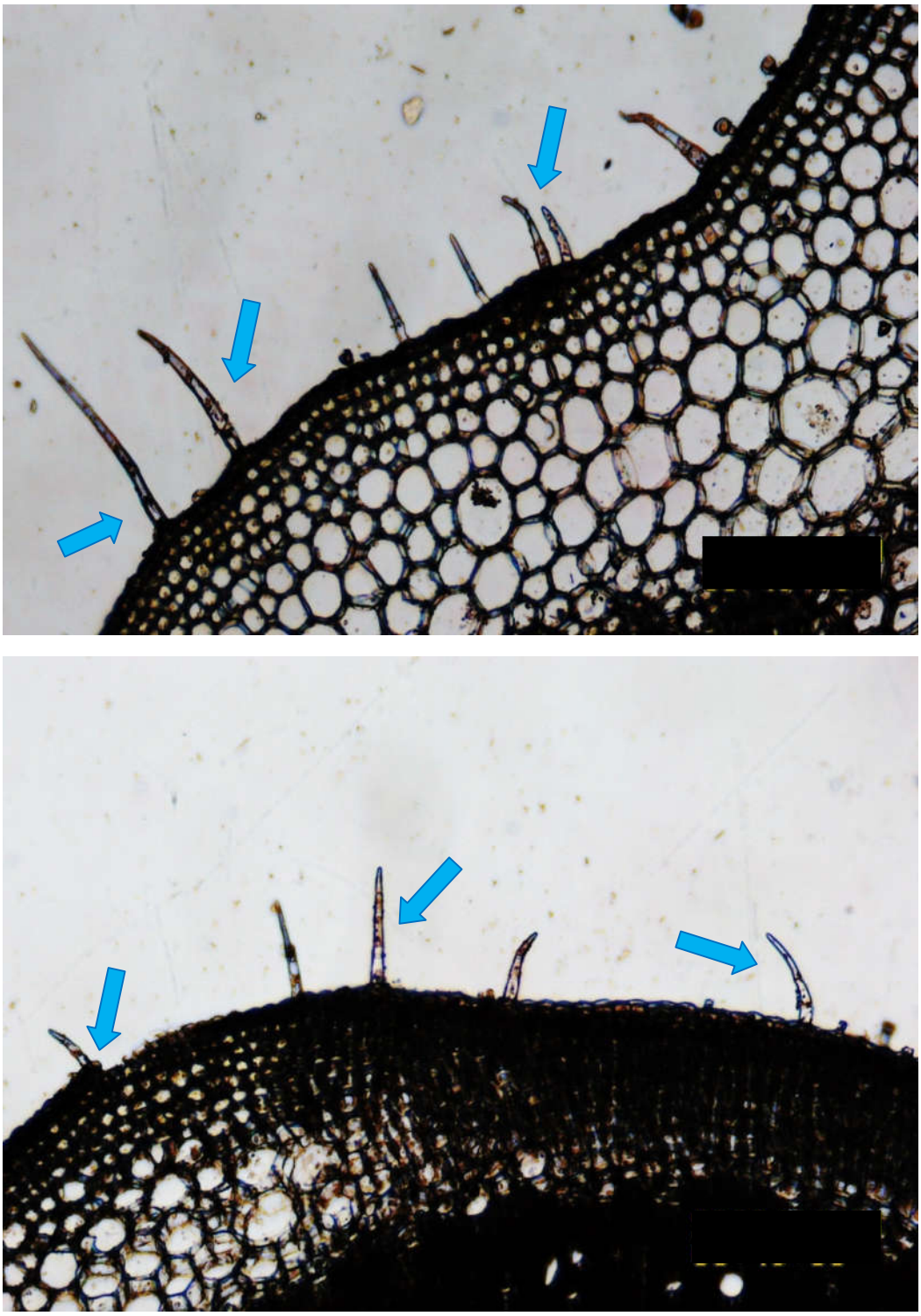

Figure 10. Transverse stem sections of waterlogged sesame plants showing adventitious root initiation. Arrows in blue indicates adventitious roots.

4.4 Yield and yield contributing parameters

4.4.1 Plant height

The height of plants at the time of harvesting was reduced under waterlogging condition, but not significantly except in the plants waterlogged for 6 days. A reduction of 20, 17 and 12% was recorded in sesame plants waterlogged for 6 days at vegetative, reproductive and maturity stages, respectively compared to their respective control (Figure 11).

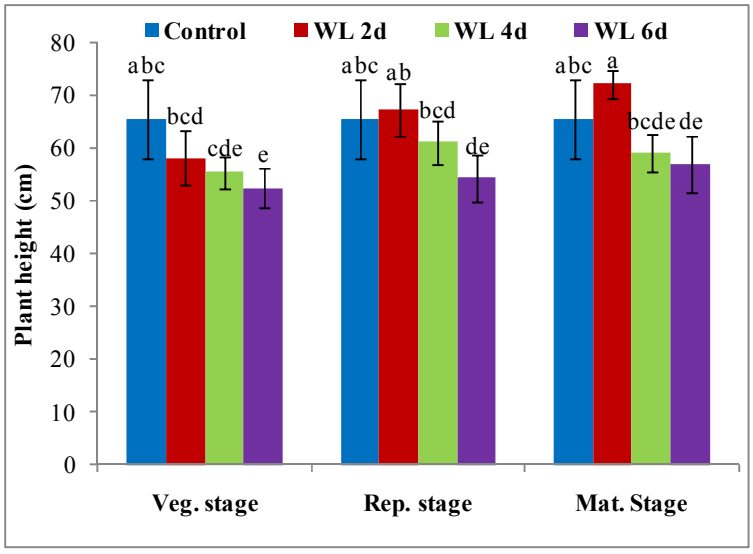

Figure 11. Effect of waterlogging on plant height of sesame at harvest. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

However, a slight increase (not significant) of plant height was observed in the plants waterlogged for 2 days either at reproductive or at maturity stage (Figure 11). The characteristics of plants recovery after short duration stress can be considered as the probable fact behind this result. Such kind of plant height recovery after waterlogging stress for 3 days was reported by Saha *et al*. (2016) in some new genotypes of sesame.

4.4.2 Number of capsule plant^{-1}

Waterlogging significantly reduced the number of capsule plant^{-1} irrespective of the stage and duration at which waterlogging was imposed. The plant waterlogged for 6 days during the reproductive stage showed the lowest number of capsule plant^{-1} (Figure 12) which is about 67% lower than the control plants. In some other experiments with sesame plants reduced number of capsule formation was also evident (Mensah *et al*., 2006; Saha *et al*., 2016).

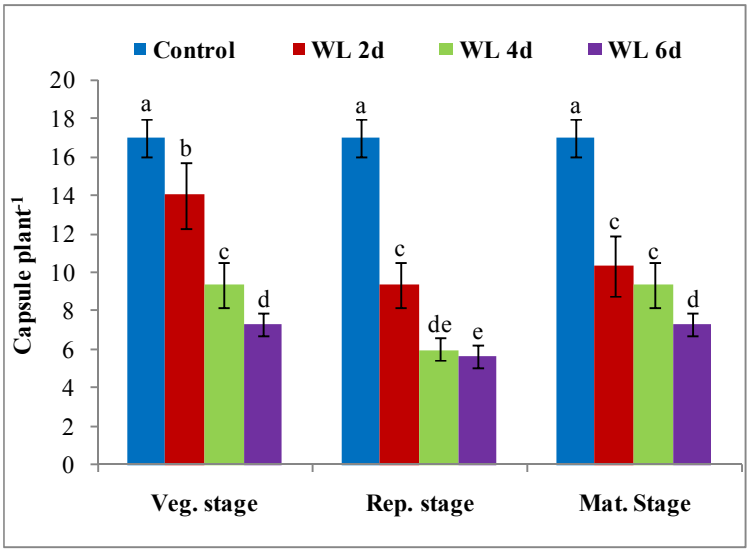

Figure 12. Number of capsule plant^{-1} affected by different durations of waterlogging in sesame crop. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

For 6 days of waterlogging at vegetative or maturity stages number of capsule plant^{-1} reduced by 59% and for 4 days it was 47% in both cases compared to the control plants (Figure 12).

4.4.3 Number of seed capsule^{-1}

Number of seed capsule^{-1} was significantly reduced in sesame plants when exposed to waterlogging for 3 days at 29 days after seedling emergence (Saha *et al.,* 2016). Same

results were observed in this experiment when waterlogging was imposed at maturity stage for 2, 4 and 6 days and at vegetative or reproductive stage for 6 days (Figure 13). However, number of seed capsule^{-1} after 2 days (46.0) and 4 days (45.3) of waterlogging stress at reproductive stage was lower than control (51.7) which was statistically similar.

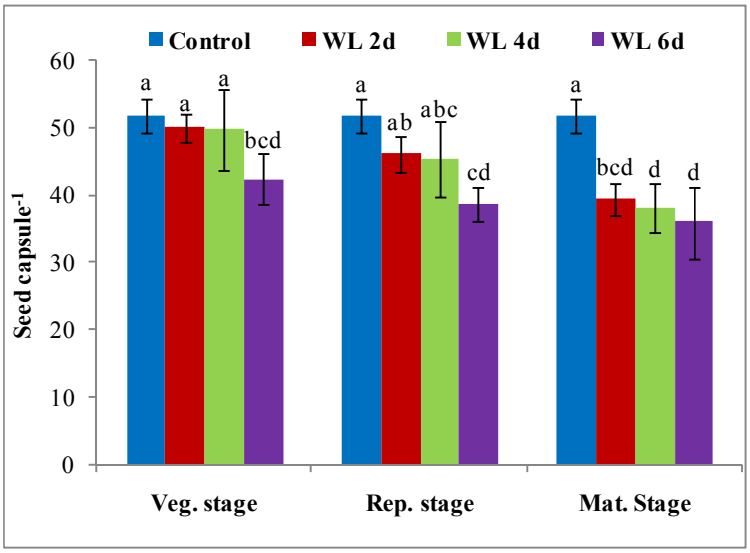

Figure 13. Number of seed capsule^{-1} affected by different durations of waterlogging in sesame crop. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.4.4 1000-seeds weight

Saha *et al*. (2016) observed that 3 days of waterlogging stress could not alter the weight of thousand seeds in three sesame genotypes which is also evident in our result for 2 days; even for 4 days when stress was imposed at maturity stage (Figure 14). The lowest value of 1000-seed weight was recorded in plants waterlogged at reproductive stage for four (2.45 g) and six (2.35 g) days, which were much lower than the control (3.12 g) plants. However, other crops like rape (Zhou and Lin, 1995),

green gram (Prasanna and Rao, 2014) and maize (Ren *et al*., 2014) have also been studied to have decreased 1000-seed weight under waterlogging stress.

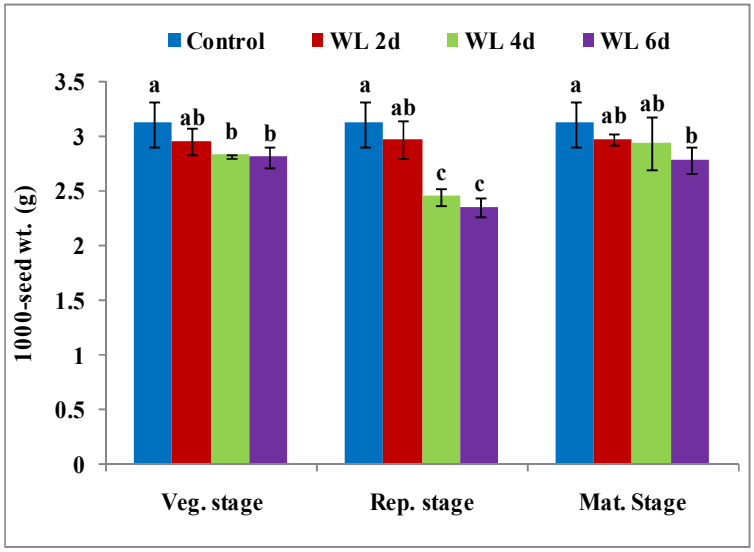

Figure 14. 1000-seed weight of sesame affected by different durations of waterlogging. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.4.5 Grain yield plant^{-1}

Highest grain yield plant^{-1} was observed in control (2.93 g) plant which was greatly reduced by waterlogging treatments irrespective of growth stages. Lowest value of grain yield plant^{-1} was recorded in plants waterlogged for 6 days (0.70 g) at reproductive stage (Table 2). Similar results were reported by Sarkar *et al*. (2016) and Saha *et al*. (2016).

4.4.6 Stover yield plant^{-1}

Stover yield plant^{-1} also decreased significantly under waterlogging stress compared to well-drained control plants. The lowest value of stover yield was recorded in plants waterlogged for 6 days at any stage. Surprisingly, 2 and 4 days of waterlogging stress could not reduce plant height significantly, but reduced the stover yield to a higher

extent. However, stover yield plant^{-1} in 2 days and 4 days of waterlogged plants were statistically similar in case of any growth stages (Table 2).

Table 2. Effect of waterlogging on grain yield plant^{-1}, stover yield plant^{-1}, biological yield plant^{-1} and harvest index of sesame

Treatments		Grain yield plant^{-1} (g)	Stover yield plant^{-1} (g)	Biological yield plant^{-1}(g)	Harvest index (%)
Control		2.93a	14.74a	17.67a	16.63a
Vegetative stage	WL 2d	2.02b	10.31c	12.33bc	16.48a
	WL 4d	1.74c	9.39cde	11.13de	15.76ab
	WL 6d	1.21e	07.79f	9.0f	13.49bc
Reproductive Stage	WL 2d	1.34de	11.99b	13.33b	10.08d
	WL 4d	0.96f	12.04b	13.0b	7.39e
	WL 6d	0.70g	08.14f	8.83f	8.0de
Maturity Stage	WL 2d	1.48d	9.85cd	11.33cd	13.05c
	WL 4d	1.33de	8.67def	10.0ef	13.27c
	WL 6d	0.93f	8.40ef	09.33f	10.06d
$LSD_{(0.05)}$		**0.083**	**0.59**	**0.56**	**1.11**
CV (%)		**6.97**	**7.14**	**5.96**	**11.00**

Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.4.7 Biological yield plant^{-1}

Biological yield means the sum total of grain yield and stover yield plant^{-1} which was also significantly lessened due to waterlogging stress. Like grain yield values, lowest value of biological yield plant^{-1} was also recorded at reproductive stage of

waterlogged treatment. Longest duration of waterlogging resulted lowest yield of sesame at all stages of stress exposure (Table 2).

4.4.8 Harvest index

Harvest index is calculated by dividing the grain yield with biological yield and it is used in agriculture to quantify the yield of a crop against the total amount of biomass produced. Waterlogging has significant effects on harvest index of sesame mostly at reproductive stage. Lowest value of harvest index was recorded in plants waterlogged at reproductive stage for four (7.39%) and six (8%) days (Table 2).

Yield is a result of the integration of metabolic reactions in plants. Any factor that influences this metabolic activity at any period of plant growth can affect the yield. Waterlogging stress has shown mostly negative effects on yield attributes (number of capsule plant^{-1}, number of seed capsule^{-1}, grain yield plant^{-1}, stover yield plant^{-1} and biological yield) of sesame plants at different stages and durations. With increasing durations of waterlogging the damage effects got higher and at the reproductive stage it was most prominent. Plant height and 1000-seed weight was not much affected by shorter duration of stress but the longest duration (6 days) of waterlogging reduced all yield attributes including these significantly. Such negative effects of waterlogging stress on yield of sesame were also proved in earlier studies (Mensah *et al*., 2006; Sarkar *et al*., 2016; Saha *et al*., 2016).

Experiment-2

4.5 Physiological parameters

4.5.1 Relative water content

A decrease in leaf RWC indicates limited water availability for cell expansion (Katerji *et al.,* 1997). Leaf wilting symptoms were observed under the waterlogging stress because of the reduction in the leaf RWC (Figure 15). This study shows a significant reduction of leaf RWC in all the plants waterlogged at vegetative stage compared to their respective control. The reduction rate was observed lowest in the plants waterlogged for 2 days and gradually increasing with the duration of waterlogging (Figure 15). Plants waterlogged for up to 8 days showed the lowest RWC (75.13%) while it's respective control had 90% leaf RWC. Similar reduction in leaf RWC has been reported under flooding stress in pineapple by Min and Bartholomew (2005) and in mung bean by Kumar *et al.* (2013).

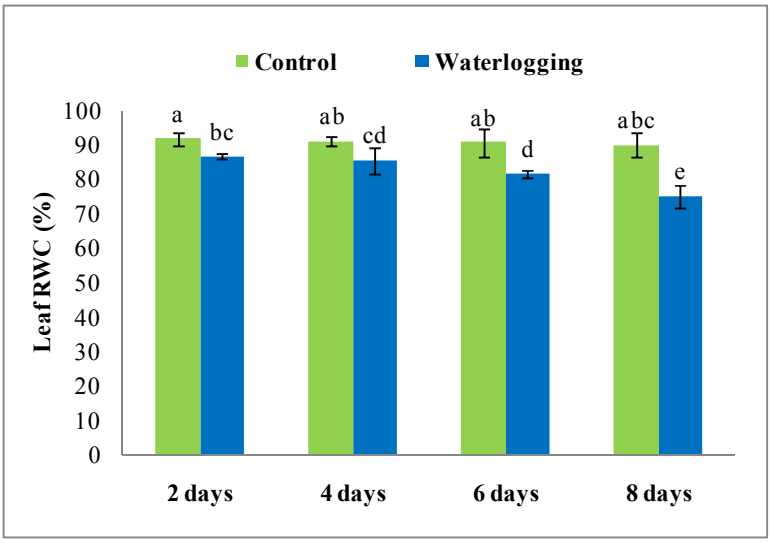

Figure 15. Changes in leaf RWC of sesame plants waterlogged at vegetative stage. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.5.2 Chlorophylls and carotenoids contents

Waterlogging has drastically reduced the contents of photosynthetic pigments in sesame plants which were also observed as the yellowing of leaves followed by chlorosis. Under waterlogging, yellowing of the plant might be due to reduction in leaf nitrogen (Bacanamwo and Purcell, 1999), nodulation and N fixation and production of toxic substances such as nitrites and sulphides which move from the soil through roots to the leave if carried upward in large quantities (Ezin *et al,*. 2010). In addition, waterlogging results in reduced soil nitrogen through rapid volatilization and denitrification (Rasaei *et al.,* 2012b). In our study, it has been observed that sesame seedlings under waterlogged condition tend to reduce their content of photosynthetic pigments with increasing duration of waterlogging (Figure 16). Chlorophyll *a* content was recorded as lowest in the plants waterlogged for 8 days which were statistically similar with the plants waterlogged for 6 days (Figure 16A). Same trend of reduction was observed for total chl and carotenoids content (Figure 16C, D). Chlorophyll *b* content was also lower in all waterlogged plants compared to their respective controls, but among the plants waterlogged for 2, 4 and 6 days, it was statistically similar (Figure 16B). Total chl or chl (*a*+*b*) was found to have a sharp decline with increasing duration of waterlogging up to 6 days, which was statistically similar with 8 days waterlogging (Figure 16C). Similarly, reduction in total chlorophyll content as a result of flooding has been reported in wheat (Collaku and Harrison, 2002), maize (Prasad *et al.,* 2004), sesame (Mensah *et al.* 2006; Wei *et al.,* 2013), onion (Yiu *et al.,* 2008) and mungbean (Kumar *et al.,* 2013).

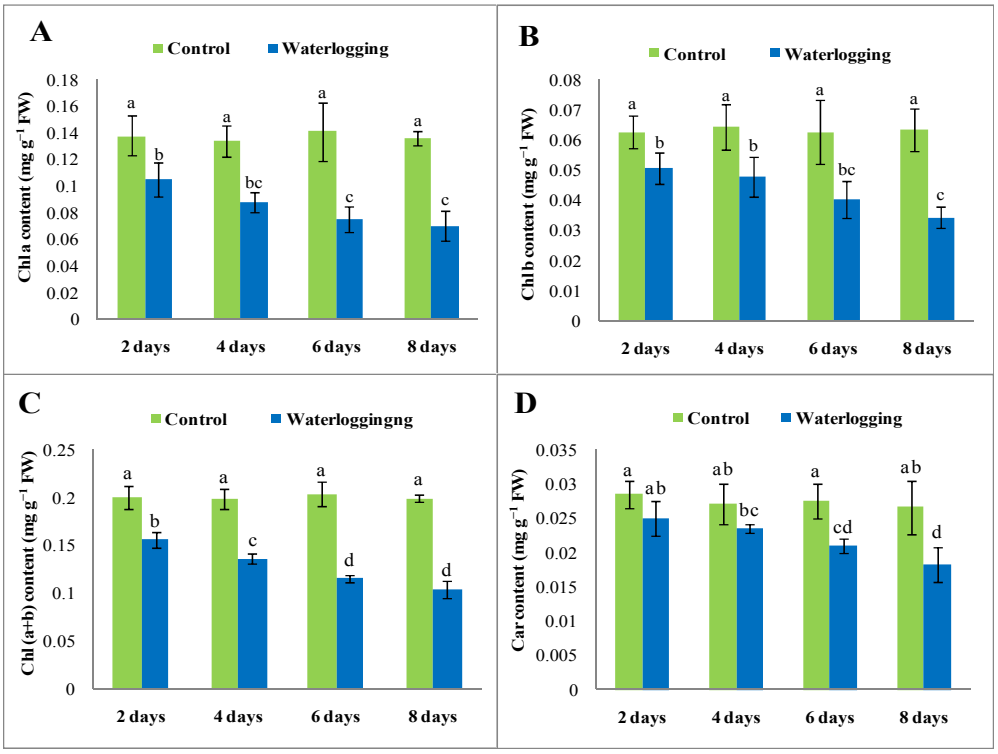

Figure 16. Contents of (A) chl *a*, (B) chl *b*, (C) total chl and (D) carotenoids of sesame leaves affected by waterlogging stress at vegetative stage. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

Higher levels of carotenoids are considered to have some protective roles against waterlogging stress in tolerant type species of plants (Kumar *et al.*, 2013). Our study showed that carotenoids content was significantly reduced in the plants waterlogged for 6 days or longer periods (Figure 16D) which denotes the lower tolerance of this cultivar under waterlogging stress. The result was supported by the findings of both Kumar *et al.* (2013) in waterlogged mung bean plants and Tiryakioğlu *et al.* (2015) in bread-wheat plants exposed to anaerobic condition.

4.6 Oxidative stress indicators

4.6.1 Lipid peroxidation (MDA content)

Lipid peroxidation destroys the integrity and function of cell membranes as well as results in cell death (Panda and Choudhury, 2005). As an indicator of lipid peroxidation, MDA content is measured to determine the degree of oxidative stress in stressed plants. To determine the degree of oxidative stress, lipid peroxidation is considered as an important index and it is found that MDA content increases with the extent of oxidative stress caused by abiotic stress including waterlogging (Hasanuzzaman *et al.,* 2012b). The overproduced ROS such as $^1O_2$, $O_2^{\bullet-}$, H_2O_2 and $OH^{\bullet}$ are highly reactive and by their subsequent reactions MDA is formed as an oxidation product (Gill and Tuteja, 2010).

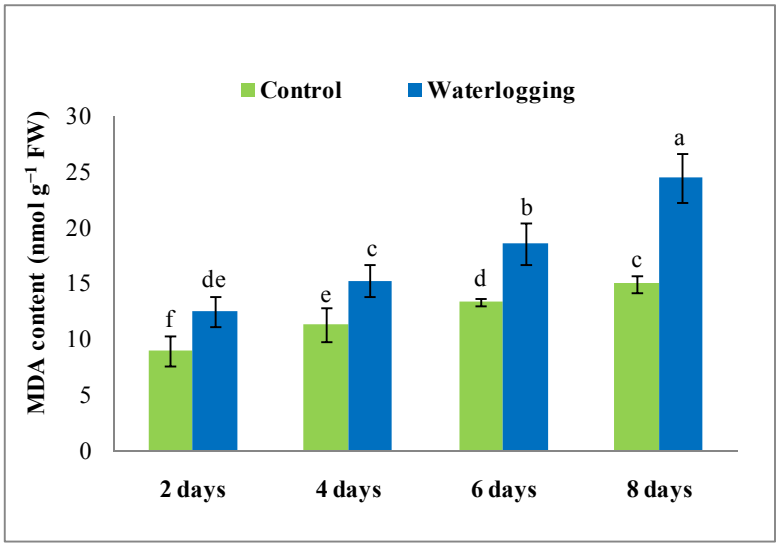

Figure 17. MDA content of sesame leaves affected by waterlogging stress at vegetative stage. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

In this study, a sharp increase of MDA content was noticed in all waterlogged plants which were significantly higher compared to their respective control (Figure 17). The

rate of MDA production has been recorded to increase in a time-dependent manner. The highest MDA was recorded in plants waterlogged for 8 days (39% higher than control) which is statistically similar with the plants waterlogged for 6 days. A similar time-dependent enhancement of MDA content in sesame seedlings under waterlologging condition for 2 days (Xu *et al.,* 2012), 3 days (Saha *et al.* 2016) and 9 days (Wei *et al.,* 2013) was observed. Zhang *et al.* (2007) and Yin *et al.* (2009) also reported the same in case of chrysanthemum cultivars and barley, respectively.

4.6.2 H_2O_2 content

H_2O_2 is noteworthy because it readily permeates membranes and therefore is not compartmentalized in the cell. H_2O_2 may inactivate enzymes by oxidizing their thiol groups (Hasanuzzaman and Fujita, 2011). Higher accumulation of H_2O_2 indicates the oxidative stress in plants. Enhanced production of H_2O_2 under waterlogging stress has been reported in wheat (Zheng *et al.,* 2009), pigeon pea (Sairam *et al.,* 2009) and Onion (Yiu *et al*., 2009). Our study also showed a significant increase of H_2O_2 contents in all sesame seedlings under waterlogged condition. Like lipid peroxidation levels, 8 days waterlogged plant showed the highest H_2O_2 content which was statistically similar with 6 days waterlogged plants (Figure 18). And also the H_2O_2 content of 4 days waterlogged plants were statistically similar with plants waterlogged for 2 days (Figure 18). However, H_2O_2 also showed a time-dependent manner of increase under waterlogged condition.

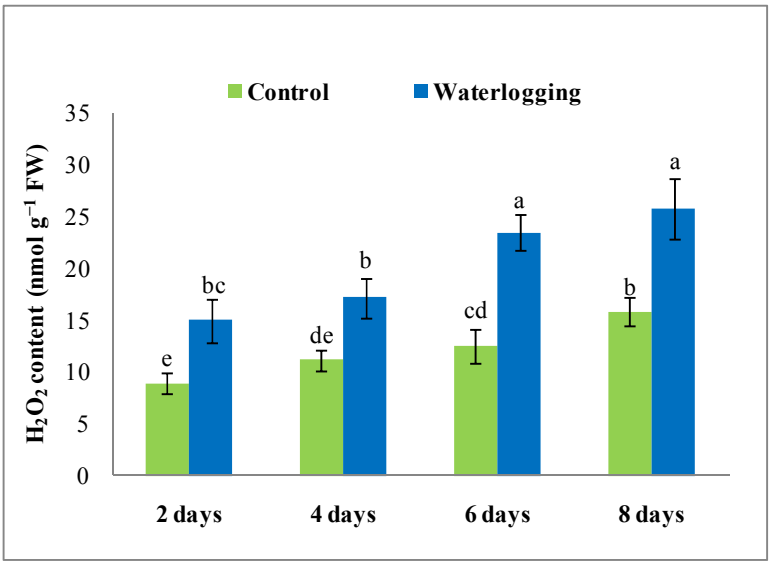

Figure 18. H_2O_2 content of sesame leaves affected by waterlogging stress at vegetative stage. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.6.3 Proline content

Proline acts as osmoprotectant molecule maintaining and improving water status of plants. Free proline also acts as antioxidant; thus protects cell from free radical damage, it maintains cellular environment favorable synthesis for biomolecules having roles in stress adaptation. Increase of Pro content under aboitic stress conditions is considered to be involved in osmoregulation and restoration of water status and increase of leaf RWC of plants (Nahar *et al.,* 2016a). But as shown in Figure 19, Pro content could not increase in the sesame cultivar and is significantly reduced in plants waterlogged for 6 and 8 days. This may be due to the lower tolerance or higher susceptibility of this sesame cultivar to waterlogging induced osmotic stress.

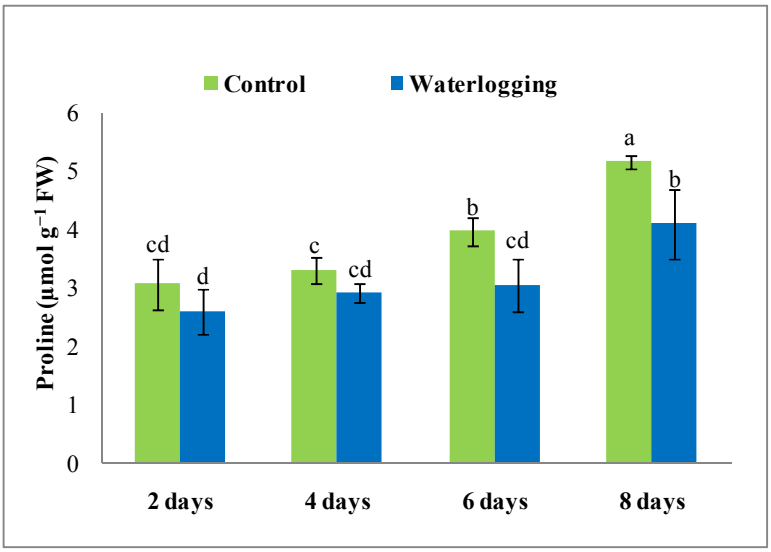

Figure 19. Proline content of sesame leaves affected by waterlogging stress at vegetative stage. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

However, a significant increase of Pro content in seedlings waterlogged for 8 days compared to the other waterlogged plants possibly denotes an attempt to restore the osmotic stress in plants (Figure 19). Because tolerant sesame genotypes showed higher Pro content under waterlogged condition compared to the sensitive type (Xu *et al.,* 2012). Onion seedlings waterlogged for up to 9 days also showed reduced Pro content (Yiu *et al.,* 2009) which supports the findings of this experiment.

4.6.4 Ascorbate and glutathione contents

To negate the detrimental effects of ROS, plants are equipped with an array of non-enzymatic scavengers and antioxidant enzymes acting in concert to alleviate cellular damage under oxidative stress conditions. AsA is one of the most abundant non-enzymatic antioxidant, serving as a major contributor to the cellular redox state and protecting plants against oxidative damage (Smirnoff, 2000). It is the substrate of APX which is a critical component of the AsA–GSH cycle for H_2O_2 detoxification

(Nakano and Asada, 1981; Dalton *et al*., 1986). In our present study, AsA content significantly decreased with increasing stress duration (Figure 20) which corroborated other reports with different abiotic stresses (Hasanuzzaman *et al.,* 2011a, b). Though irregular changes of AsA content has been reported in both tomato and eggplant genotypes which were exposed to waterlogging for 12, 24, 36, 48, 60 and 72 hrs (Lin *et al.,* 2004).

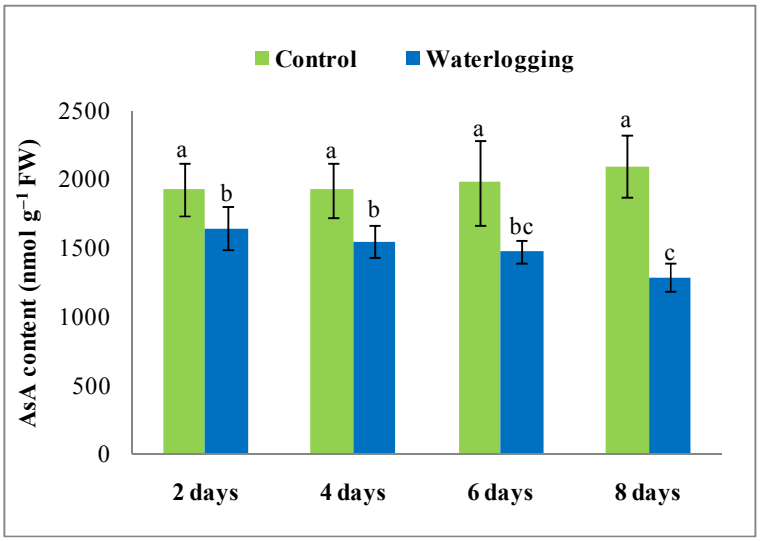

Figure 20. Ascorbate (AsA) content of sesame leaves affected by waterlogging stress at vegetative stage. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

Glutathione (GSH) is a non-protein, sulfhydryl-containing molecule, and having vital role in the stress defense systems (Gill and Tuteja, 2010). Like AsA, GSH also plays a pivotal role in preventing cell against oxidative damage by equilibrating redox status. GSH can participate not only in scavenging H_2O_2 through the AsA–GSH cycle but also in direct reactions with other ROS (May *et al*., 1998). The increased level of the GSH pool is generally regarded as a protective response against oxidative stress. As shown in Figure 21A, GSH content significantly increased in the stressed plants when waterlogged for 4 or more days. But there was no significant difference between the

plants waterlogged for 6 and 8 days and it may be because of the higher GSSG contents as GSH participates in ROS scavenging and converts into GSSG.

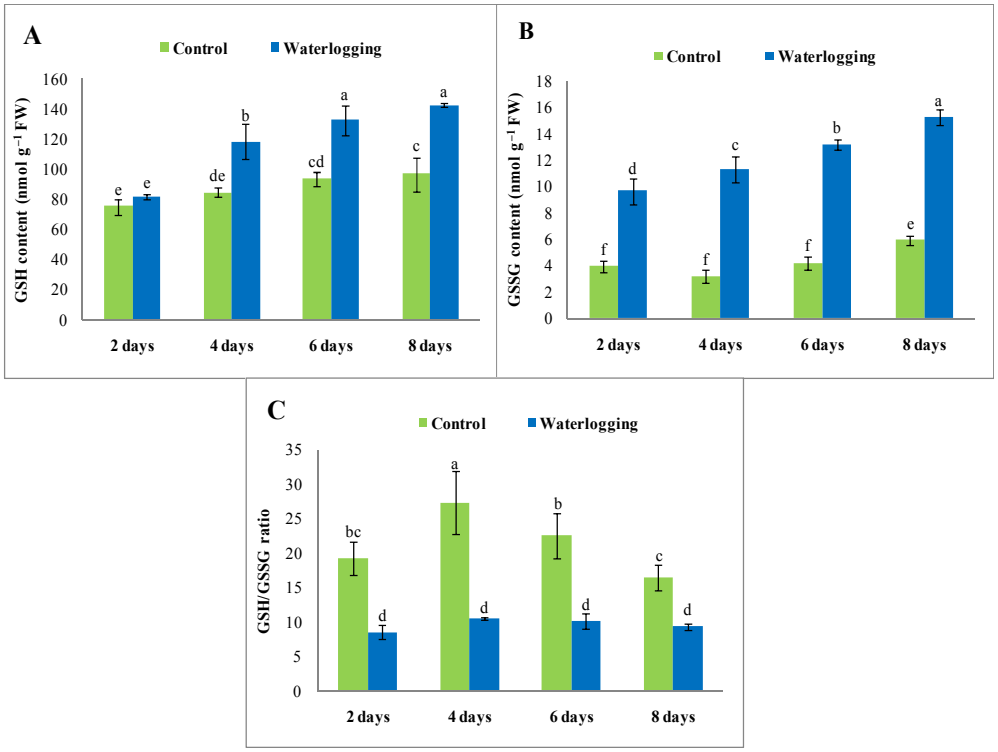

Figure 21. (A) GSH, (B) GSSG contents and (C) GSH/GSSG of sesame leaves under waterlogged condition for different durations at vegetative stage. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

Significant increase of GSSG content in all the waterlogged plants compared to their respective control has also been observed (Figure 21B). These results of both GSH and GSSG were alike the result of plants exposed to other abiotic stresse like salinity (Hasanuzzaman *et al*., 2011a, b), high temperature (Hasanuzzaman *et al*., 2014) and drought (Nahar *et al*., 2015a). Notable increase of both the GSH and GSSG contents occurred in a time-dependent manner of waterlogging which corroborated the result of Lin *et al.* (2004). However, different duration of waterlogging tend to have no

significant changes of the GSH/GSSG ratio of sesame plants (Figure 21C) which supports the results reported by Lin *et al.* (2004) in eggplant seedlings.

4.6.5 Activities of antioxidant enzymes

4.6.5.1 CAT activity

Catalase (CAT) is a tetrameric heme-containing enzyme that uses H_2O_2 as a substrate and converts it to H_2O and O_2 (Sánchez-Casas and Klessig, 1994). So, it is considered as a vital enzyme for ROS detoxification. This study observed a gradual decline of CAT activity in waterlogged plants with duration increasing (Figure 22). Higher production of toxic H_2O_2 compared to the control plants may be the reason of this diminished CAT activity. With same durations of waterlogging higher activity of CAT has been observed in both tolerant and sensitive genotypes of sesame seedlings (Wei *et al.,* 2013). However, waterlogging induced reduction of CAT activity has also been reported in barley (Zhang *et al.,* 2007), wheat (Tan *et al.,* 2008), onion (Yiu *et al.,* 2009) and sesame (Xu *et al.,* 2012).

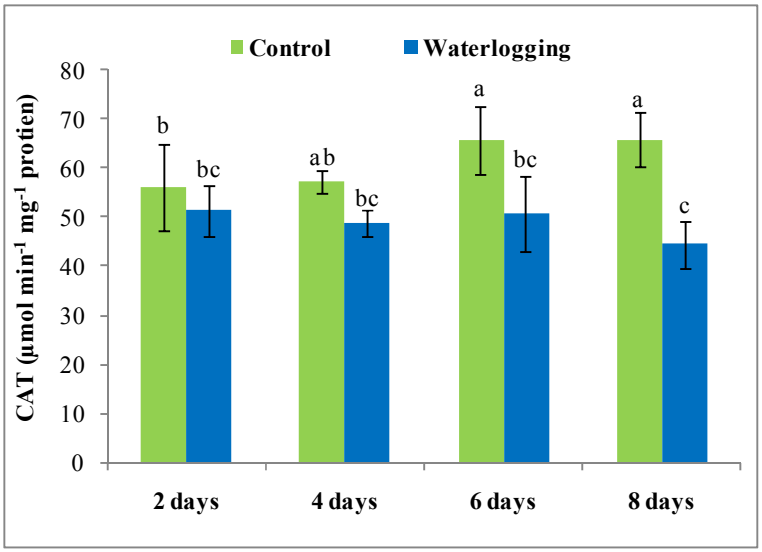

Figure 22. Activity of catalase (CAT) enzyme in sesame leaves under waterlogged condition for different durations at vegetative stage. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

In the previous studies of Sairam *et al*. (2009) with pigeon pea and Bin *et al*. (2010) with maize, it has been observed that the CAT activity is likely to increase in the tolerant types of cultivars as well as decreasing in the sensitive types which denotes the sensitivity of our sesame cultivar to waterlogging stress.

4.6.5.2 APX activity

Ascorbate peroxidase (APX) is the first line enzyme of AsA-GSH cycle. The enzymes of the AsA-GSH cycle (APX, MDHAR, DHAR and GR) readily and efficiently catalyze ROS detoxification with the help of the vital components AsA and GSH; after scavenging ROS, AsA and GSH are recycled (Li *et al*., 2010; Pang and Wang, 2010; Apostolova *et al*., 2011). The APX catalyzes the reduction of H_2O_2 to H_2O by using AsA (Wang *et al*., 2006; Xu *et al*., 2008). Waterlogged plants of sesame (Wei *et al.,* 2013; Saha *et al*., 2016), maize (Bin *et al.,* 2010), eggplants (Lin *et al.,* 2004) have been reported to have higher APX activity compared to control which support the results of our study (Figure 23). However, lessened activity of APX in onion plants under waterlogged condition has also been documented (Yiu *et al.,* 2009).

As shown in Figure 23, waterlogging has significantly increased the APX activity in plants waterlogged for 6 and 8 days which are both statistically similar and much higher compared to their respective control.

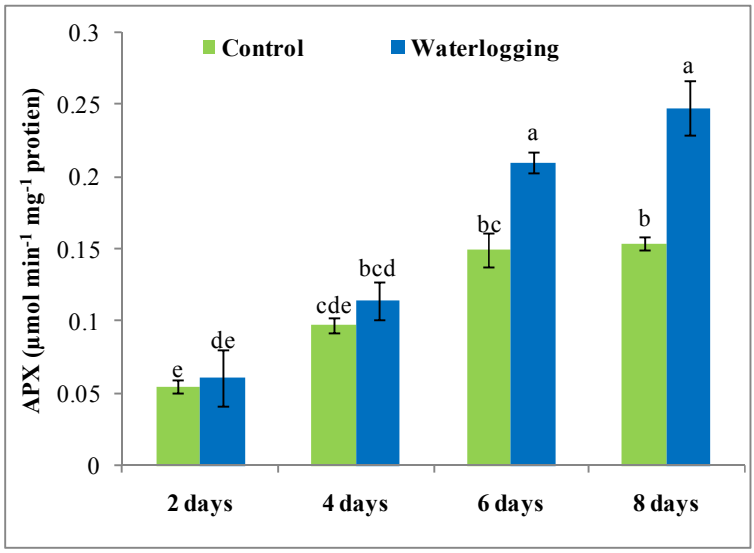

Figure 24. Activity of ascorbate peroxidase (APX) enzyme in sesame leaves under waterlogged condition for different durations at vegetative stage. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.6.5.3 MDHAR activity

Monodehydroascorbate reductase (MDHAR) is another important enzyme of AsA-GSH cycle. The univalent oxidation of AsA leads to the formation of MDHA. If MDHA is not reduced again to AsA by MDHAR, it will spontaneously disproportionate into AsA and DHA. The regeneration of AsA could be regulated in this cycle mainly by NADPH-dependent MDHAR activity (Mittova *et al.,* 2000) and thus it is crucial for AsA regeneration and essential for maintaining a reduced pool of AsA (Martínez and Araya, 2010) . Although there are also a few reports about MDHAR activity in other physiological processes that are related to oxidative stress, research on different crops under environmental stresses revealed the regulatory role of MDAHR during oxidative stress tolerance and acclimation (Mittova *et al.,* 2003; Hasanuzzaman *et al.,* 2012a).

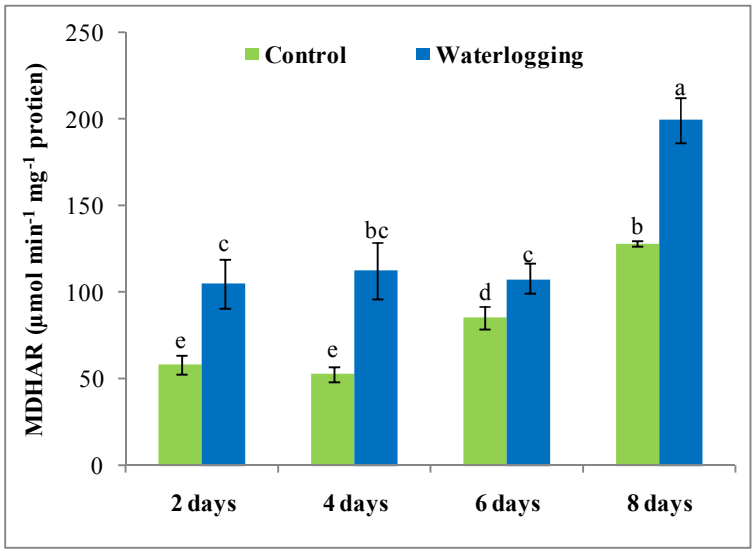

Figure 24. Activity of monodehydroascorbate reductase (MDHAR) enzyme in sesame leaves under waterlogged condition for different durations at vegetative stage. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

Sesame plants under this experiment showed significantly higher MDHAR activities under waterlogging stress compared to the same aged well-drained plants, but unlike APX it showed no time-dependent increase of activity, rather a sudden increase of 55% at 8 days of waterlogging treatment, compared to the control plants (Figure 24). This may be due to the non-significant changes in the AsA contents of the same plants (Figure 20). No previous report has been established regarding the MDHAR activities under waterlogging stress.

4.6.5.4 DHAR activity

Dehydroascorbate reductase (DHAR) is an equally important enzyme as MDHAR in regulating the level of AsA and its redox state under oxidative stress (Eltayeb *et al.,* 2007). DHAR is also a key component of the AsA recycling system (Martínez and Araya, 2010) which regenerates AsA from the oxidized state (DHA) and regulates the

cellular AsA redox state (Hasanuzzaman *et al.,* 2012a). It is thus crucial for tolerance to various abiotic stresses leading to the production of ROS.

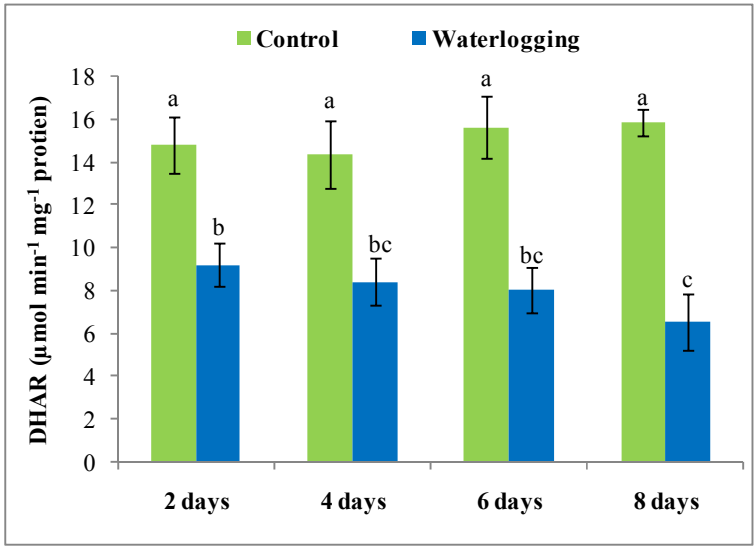

Figure 25. Activity of dehydroascorbate reductase (DHAR) enzyme in sesame leaves under waterlogged condition for different durations at vegetative stage. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

There are several studies revealing the diminished activities of DHAR under various abiotic stresses like salinity (Nahar *et al.,* 2015b), drought (Nahar *et al.* 2015a), high temperature (Nahar *et al.* 2015c) and heavy metal (Mahmud *et al*., 2017) stress; but there are hardly any record of DHAR activity under waterlogging stress yet documented. Our experiment also showed a significant reduction of DHAR activities by 38, 42, 49 and 58.5% in plants waterlogged for 2, 4, 6 and 8 days respectively compared to the same aged well-drained plants. However, the highest reduction occurred in the plants waterlogged for longest duration (8 days) which is statistically higher than the other duration (2 to 6 days) of stressed plants (Figure 25).

4.6.5.5 GR activity

Glutathione reductase (GR) is a potential enzyme of the AsA-GSH cycle and plays an essential role in the defense system against ROS. This experiment showed significantly lower GR activities in sesame plants when waterlogged for 6 or 8 days, whereas 2 and 4 days of waterlogging treatment could not affect the GR activity to any context (Figure 26). Such reduction of GR in higher levels of stress has been also demonstrated by Hasanuzzaman *et al*. (2011a) in salinity stress. In case of waterlogging stress, a time-dependent reduction of GR activities has been demonstrated in barley up to 18 days of stress treatment, though it was always higher than the control plant (Zhnag *et al.,* 2007).

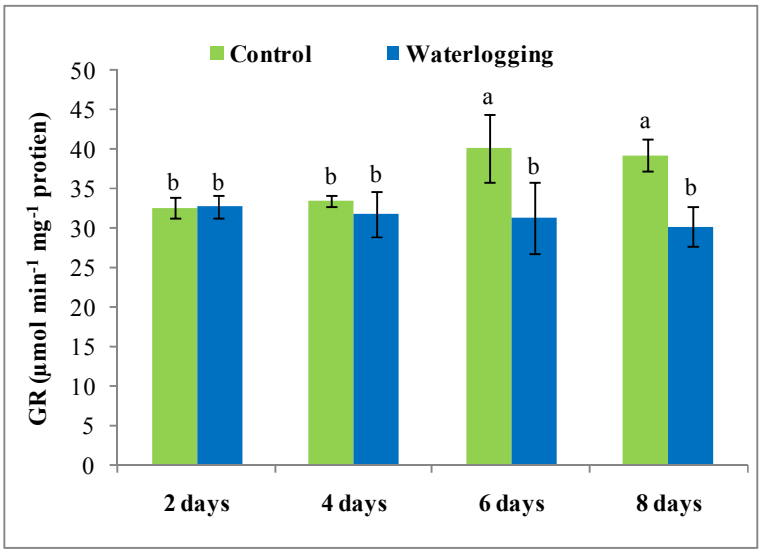

Figure 26. Activity of glutathione reductase (GR) enzyme in sesame leaves under waterlogged condition for different durations at vegetative stage. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

However, Yiu *et al*. (2009) also observed reduced GR activity in onion plants waterlogged for 10 days. Though, increased GR activity is reported to confer stress tolerance and has the ability to alter the redox state of important components of the

ETC, there is hardly any report showing the difference of waterlogging induced GR activity in tolerant and sensitive crop species.

4.6.5.6 GPX activity

Glutathione peroxidase (GPX) is a foremost cellular enzyme capable of repairing membrane lipid peroxidation and is an important protectant against oxidative membrane damage (Kühn and Borchert, 2002). GPX functions both as a H_2O_2-detoxifying agent and an oxidative signal transducer (Hasanuzzaman *et al.,* 2012a). Very few investigations have been documented about the effect of waterlogging stress on GPX activity. In this experiment, GPX activities increased in a time-dependent manner with increasing duration of waterlogging (Figure 27).

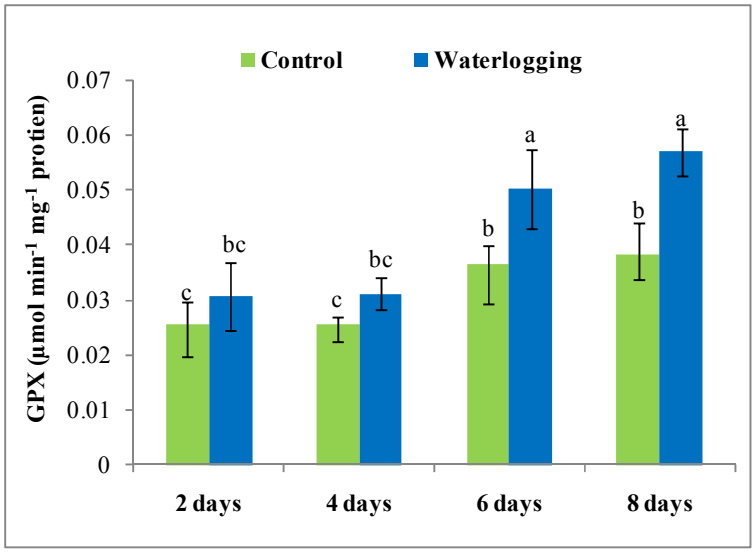

Figure 27. Activity of glutathione peroxidase (GPX) enzyme in sesame leaves under waterlogged condition for different durations at vegetative stage. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

Plants waterlogged for 6 days and 8 days both showed significantly higher (38 and 47% respectively) GPX activities compared to their respective control (Figure 27).

Similar increases of GPX activity were demonstrated by Saha *et al.* (2016) in sesame seedlings exposed to 3 days of waterlogging treatment.

4.6.6 Methylglyoxal (MG) and glyoxalase system enzymes

Methylglyoxal (MG) is a cytotoxic compound, normally present in a lower amount in plant cells, but increases several fold under stress conditions depending on stress intensity and duration (Yadav *et al.,* 2005, 2008; Hasanuzzaman *et al.*, 2017). Therefore, upregulation of the glyoxalase system is crucial for plants to build up stress tolerance against toxic MG-induced oxidative stress (Yadav *et al*., 2005, 2008). The glyoxalase system consists of two vital enzymes, Gly I and Gly II, which can detoxify MG in a two-step reaction. In the first step, the Gly I enzyme converts MG to SLG, where GSH acts as a co-factor. In the second step, Gly II transforms SLG to D-lactate, where GSH is recycled (Hasanuzzaman *et al.*, 2017b; Mahmud *et. al.,* 2017). Several other reports demonstrated the modulation of glyoxalase system under different abiotic stresses like high temperature (Nahar *et al.,* 2015c), salinity (Rahman *et al.*, 2016), drought (Nahar *et al.,* 2015a) and heavy metal (Mahmud *et al.,* 2017). But, there is no previous study demonstrating the effect of waterlogging stress on methylglyoxal detoxification system. In this new study, it was observed that when sesame seedlings were exposed to waterlogging condition, MG content increased significantly compared to their respective control. The longer the plants were under waterlogged condition, the higher the generation of MG was recorded. An increase in MG content by 60, 42, 46 and 47% was observed in sesame seedlings waterlogged for 2, 4, 6 and 8 days, respectively compared to their respective control plants (Figure 28).

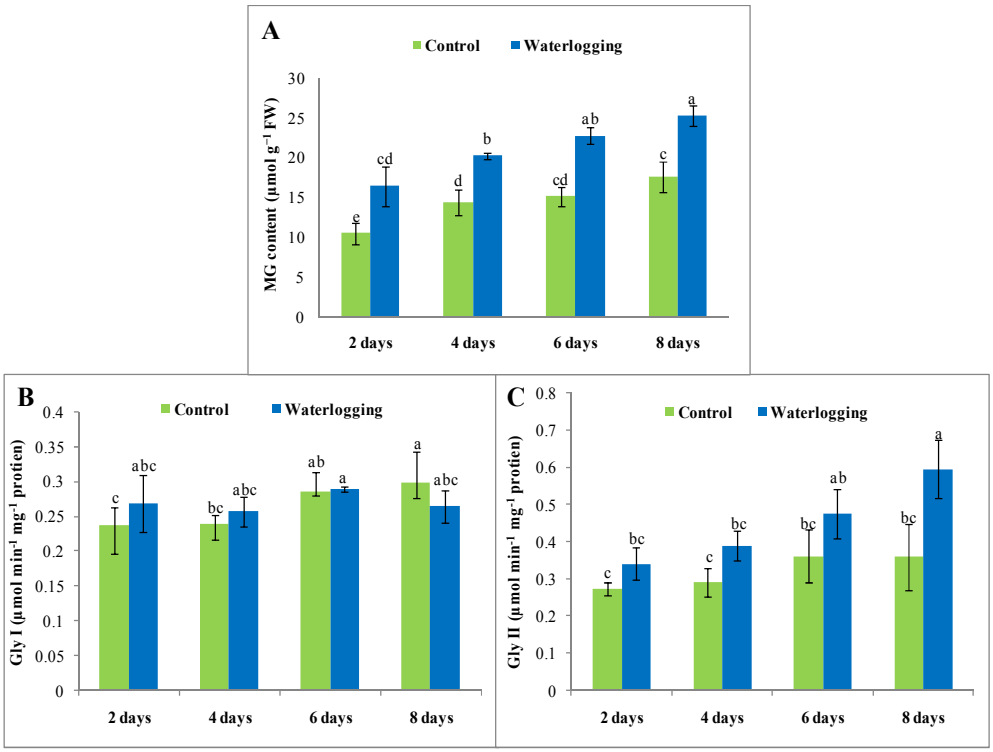

Figure 28. (A) MG content, (B) Gly I and (C) Gly II activities in sesame leaves under waterlogged condition for different durations at vegetative stage. Mean (±SD) was calculated from three replicates for each treatment. Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD test

On the other hand, the two related enzymes- Gly I and Gly II showed divergent mode of activities under the same condition. The activity of Gly I was higher at 2 days of waterlogging which slightly declined at 4 days and then had a subsequent increase at 6 days of waterlogging. However, it again decreased sharply at 8 days of waterlogging which may be due to the excessive production of the cytotoxic MG and lower production of GSH compared to that. Unlike Gly I, Gly II activity was enhanced with increasing duration of stress with the highest activity in the plants waterlogged for 8 days (Figure 28). The possible reason underlying this fact is the higher generation of GSSG which is responsible for the recycling of GSH.

4.6.7 Overall mechanism of oxidative stress responses under waterlogging

Elevated MDA and H_2O_2 contents (Figure 17, 18) manifested the generation of ROS and subsequent oxidative stress in sesame plants under waterlogging stress. However, non-enzymatic AsA and GSH (Figure 20, 21) and enzymatic antioxidants (Figure 22−27) have potential roles in detoxifying these ROS and reducing the damages induced by oxidative stress. In addition, higher level of toxic MG production has been recorded which is possibly detoxified by glyoxalase enzymes activities (Figure 28). The figure below (Figure 29) indicates a possible outline of waterlogging induced oxidative stress and subsequent action of antioxidant defense and glyoxalase systems to mitigate the stress damages which need to be elucidated by further studies.

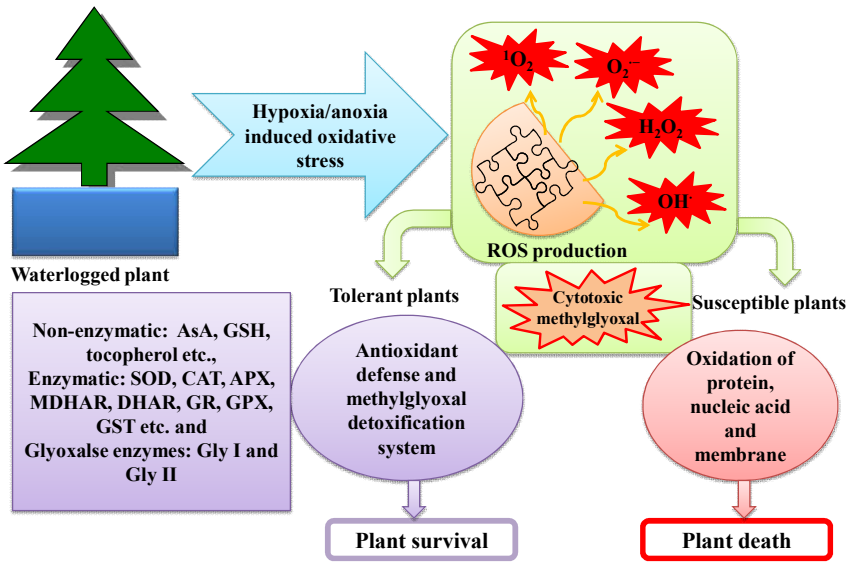

Figure 29. Oxidative stress responses under waterlogging and possible mechanism of ROS and toxic MG detoxification by antioxidant defense and glyoxalse system

Chapter 5

SUMMARY AND CONCLUSION

There were two experiments conducted at different locations and periods to investigate the responses of sesame crop to waterlogging stress. Experiment-1 was conducted inside the experimental shed of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period of April−September, 2015 where we studied the morpho-physiological, anatomical and yield attributes of sesame crop as affected by different durations of waterlogging (2, 4 and 6 days) at vegetative, reproductive and maturity stages. Later, during the period of April−September, 2016 the experiment-2 was conducted inside the greenhouse of Faculty of Agriculture, Kagawa University, Kagwa, Japan where some physiological and mainly the oxidative responses of sesame to waterlogging stress for up to 8 days were assessed.

The experiments were arranged in a Randomized Completely Block Design (RCBD) with three replications. Plastic pots were used to facilitate the development of waterlogging stress. Experiment-1 consisted of 10 treatments: control (well-drained), waterlogging at vegetative stage for 2, 4 and 6 days, at reproductive stage for 2, 4 and 6 days and at maturity stage for 2, 4 and 6 days. Expriment-2 consisted of 5 treatments: control (well-drained), waterlogging for 2, 4, 6 and 8 days at vegetative stage. Treatments were imposed at 21 DAS considering as vegetative stage, at 35 DAS considering that as reproductive stage and at 55 DAS considering that as maturity stage. There were five seedlings maintained in each pot.

In experiment-1, data were taken at the completion of stress duration for each treatment. Yield parameters were measured while harvesting at 90 DAS. In

experiment-2, data were measured after the completion of each treatment duration. Mortality rate, plant height, leaves plant^{-1}, leaf area, above ground fresh weight and above ground dry weight were measured for growth. SPAD value was measured manually with atLEAF as a physiological parameter. At harvest, plant height, capsule plant^{-1}, seed capsule^{-1}, 1000-seed weight, grain yield, stover yield, biological yield plant^{-1} and harvest index were measured to assess the effect on yield. The content of photosynthetic pigments was also measured by leaf extraction in laboratory in experiment-2. Relative water content (RWC) was also measured to understand the effect on plant water relations. Biochemical parameters including lipid peroxidation (MDA content), H_2O_2, Pro, AsA, GSH, GSSG, MG and antioxidant enzyme like CAT, APX, MDHAR, DHAR, GR, Gly I and Gly II activities were measured.

Mortality rate (%) of seedlings was the highest at vegetative stage (40%) and the lowest at maturity stage (10%) when waterlogging continued for 6 days. The highest damaging effects were observed in all plants waterlogged for 6 days at any stage of growth. Plant height was recorded to reduce mostly at maturity stage (6 days waterlogging) which was 11.5 cm shorter than the control, whereas at reproductive stage it got 8.8 cm shorter and at vegetative stage 5.4 cm for same duration of stress. Number of leaves plant^{-1} and leaf area were mostly reduced at reproductive stage and maturity stage for both 4 and 6 days of waterlogging stress. Short duration (2 days) of waterlogging had positive effect on FW and DW of sesame when imposed at maturity stage with a negligible decrease at vegetative stage, but longer durations (4 or 6 days) significantly reduced both. But, waterlogging at reproductive stage significantly reduced both FW and DW for any duration.

Though SPAD value (measure during experiment-1) gives a proportional value of the leaf chlorophyll content, we measured it by leaf extraction in laboratory also (during

experiment-2). Both SPAD units and chlorophyll contents of leaves decreased in a time-dependent manner with increasing duration of waterlogging which verifies the damaging effect of stress on leaf photosynthetic apparatus. Carotenoid content also showed similar pattern of reduction. Waterlogging results in leaf wilting which is evident from the significant reduction of RWC in waterlogged plants with lowest value in plants waterlogged for 8 days (75%). Significant reduction of Pro content at longer duration of stress (6 or 8 days) denotes the collapse of plant cells due to higher osmotic stress.

From the anatomical study of the experiment, it was evident that sesame plants formed aerenchyma in their stem cortex to capture oxygen and initiated adventitious root on the stem surface. This proved the ability to possess some adaptive mechanisms in sesame plants.

Abiotic stress induced oxidative stress is a common phenomena and waterlogging as an abiotic stress also increased the lipid peroxidation and H_2O_2 contents of sesame leaves significantly. Collating all the waterlogged plants, 8 days of waterlogging showed the highest contents of both MDA (24.44 nmol g^{-1} FW) and H_2O_2 (25.77 nmol g^{-1} FW) which gradually got higher in a time-dependent manner with the lowest value for 2 days of waterlogging. Antioxidant defense system comprises non-enzymatic antioxidants such as AsA and GSH and enzymatic antioxidants such as CAT, APX, MDHAR, DHAR and GPX etc. This study revealed waterlogging induced reduction of AsA content and enhancement of GSH content which is true for other abiotic stresses too. Activities of APX, MDHAR and GPX increased with duration of stress getting longer and CAT, DHAR and GR activities reduced in a time-dependent manner.

There was hardly any study demonstrating the generation of cytotoxic MG and the activation of glyoxalase system enzymes- Gly I and Gly II under waterlogging stress. Hence, it can be considered as the paramount finding of this experiment. Significant increase of MG content was recorded in waterlogged plants. Increased GSH content and elevated Gly II activities signify the ability to detoxify this raised content of toxic MG. But, as another important enzyme Gly I activity was not boosted in sesame; the plants got severely damaged at prolonged waterlogging stress.

From the findings of this study, it was observed that plant reacts to waterlogging stress depending mainly on the duration of stress exposure irrespective of the stage of growth. The longer the plant remains under waterlogging condition, the more the damage effects occur. However, if compared among only growth stage effects, the reproductive stage was found most sensitive to both growth and yield damages; vegetative stage to survival and maturity stage to yield attributes. However, anatomical and biochemical study of this experiment demonstrates that sesame possesses adaptive mechanisms at both morpho-anatomical and cellular levels. This can be a promising phenomenon to create further scope of investigation at gene level to discover waterlogging tolerant genes of sesame and eventually tolerant varieties.

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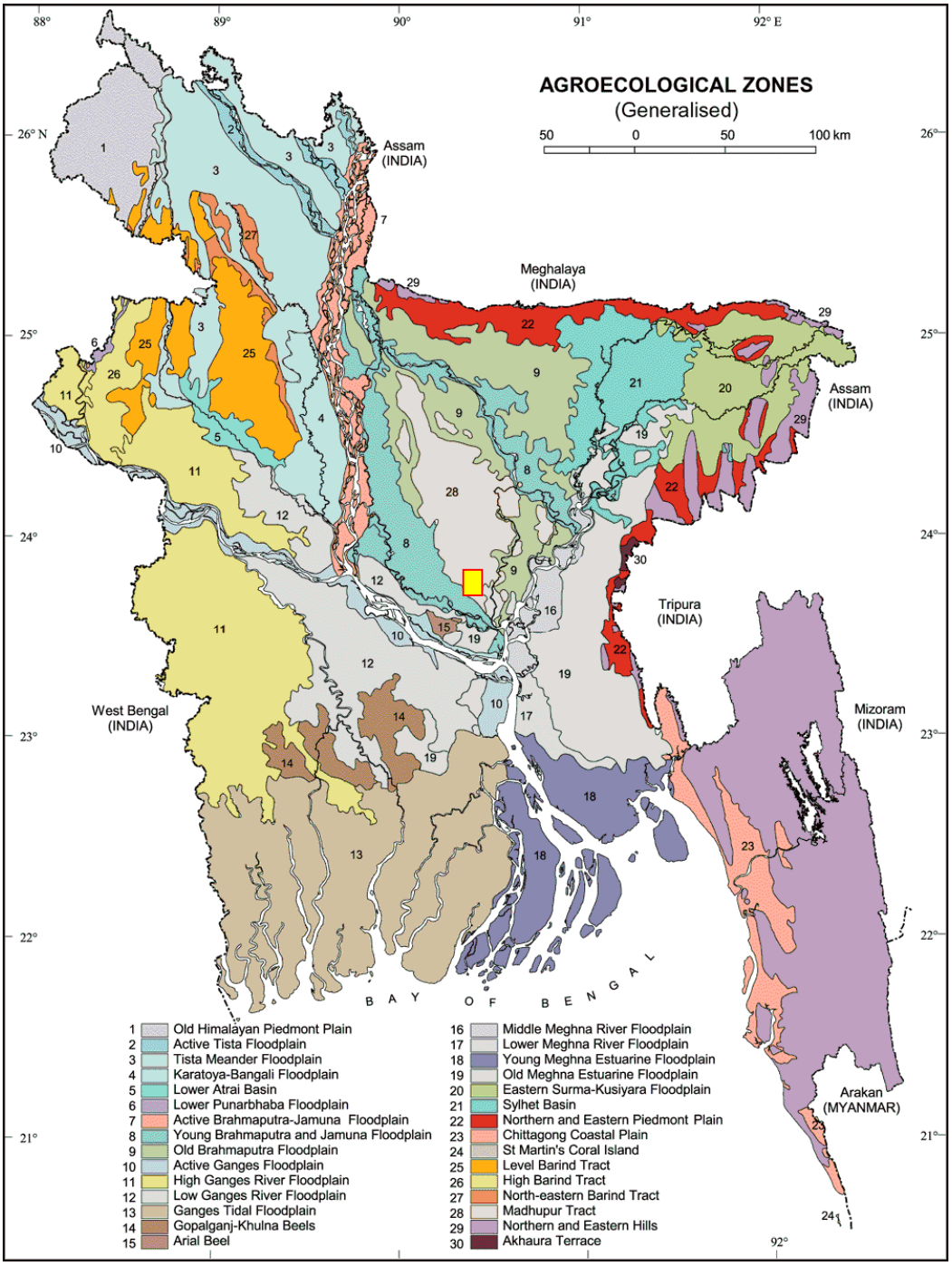
APPENDICES

Appendix I. Map showing the location of experiment-1



Appendix II. Physical and chemical properties of experiment-1 soil analyzed at Soil Resources Development Institute (SRDI), Farmgate, Dhaka

Characteristics	Values
Particle size analysis	
%Sand	27
%Silt	43
%Clay	30
Textural class	Silty-clay
pH	5.6
Organic carbon (%)	0.45
Organic matter (%)	0.78
Total N (%)	0.03
Available P (ppm)	20.00
Exchangeable K (me/100 g soil)	0.10
Available S (ppm)	45

Appendix III. Monthly average air temperature, rainfall and relative humidity of the experiment-1 site during the period from April 2015 to September 2015

Months	Air temperature (°C)		Relative humidity (%)	Total rainfall (mm)
	Maximum	Minimum		
April, 2015	32.6	23.1	67.5	181.06
May, 2015	35	25.3	70.1	176.27
June, 2015	32.7	26.5	77.3	373.38
July, 2015	31.6	25.9	81.5	674.86
August, 2015	32.6	26.9	79.1	352.02
September, 2015	32.6	26	77	320.03

Appendix IV: Mean square values

Source of variation	df	Mean square value of						
		Mortality rate	Plant height	No. of leaves plant^{-1}	Leaf area	FW	DW	SPAD value
Replication	2	49.405	5.764	11.181	3069	19.528	3.0833	5.4498
Treatment	11	658.129	543.653	672.80	1052152	843.967	42.3939	88.0444
Error	22	82.726	14.50	5.739	8106	10.134	1.4773	9.9072

Source of variation	df	Mean square value of							
		Plant height (at harvest)	No. of capsule plant^{-1}	No. of seed capsule^{-1}	1000-seed weight	Grain yield plant^{-1}	Stover yield plant^{-1}	Biological yield plant^{-1}	Harvest index
Replication	2	0.633	3.7	0.3	0.0318	.06417	5.4940	6.7443	0.2755
Treatment	9	112.774	36.996	94.9222	0.1766	1.24916	14.5323	21.5692	34.5243
Error	18	23.819	0.8852	16.9667	0.0169	0.01041	0.5236	0.4777	1.8659

Source of variation	df	Mean square value of						
		RWC	Chl a	Chl b	Chl (a+b)	Car	MDA	H_2O_2
Replication	2	10.9690	1.79×10^{-4}	4.48×10^{-5}	5.39×10^{-5}	1.34×10^{-5}	10.4179	12.223
Treatment	7	97.9051	2.69×10^{-3}	4.14×10^{-4}	5.19×10^{-3}	3.77×10^{-5}	69.0307	102.042
Error	14	7.3070	1.62×10^{-4}	4.62×10^{-5}	8.35×10^{-5}	5.05×10^{-6}	0.8770	1.972

Source of variation	df	Mean square value of					
		AsA	GSH	GSSG	GSH/GSSG	MG	Proline
Replication	2	191842	121.40	0.0593	15.092	3.1009	0.26978
Treatment	7	243681	1887.65	64.5563	147.228	69.4292	2.09111 0.10936
Error	14	11544	42.27	0.4540	3.933	2.1242	

Source of variation	df	Mean square value of							
		CAT	APX	MDHAR	DHAR	GR	GPX	Gly I	Gly II
Replication	2	21.051	0.00088	20.68	0.2004	2.0642	0.000021	0.00124	0.03912
Treatment	7	179.662	0.01407	6352.82	45.3279	40.7201	0.000387	0.00157	0.03291
Error	14	25.732	0.00097	112.83	1.6758	8.5457	0.000023	0.00073	0.00650

df – degree of freedom